2012

Evaluation of Commonly Used Antimicrobial Interventions for Fresh Beef Inoculated with Shiga Toxin–Producing *Escherichia coli* Serotypes O26, O45, O103, O111, O121, O145, and O157:H7

Norasak Kalchayanand
USDA Meat Animal Research Center, norasak.kalchayanand@ars.usda.gov

Terrance M. Arthur
USDA Meat Animal Research Center, terrance.arthur@ars.usda.gov

Joseph M. Bosilevac
USDA Meat Animal Research Center, mick.bosilevac@ars.usda.gov

John W. Schmidt
USDA-ARS, john.w.schmidt@ars.usda.gov

Rong Wang
USDA-ARS, rong.wang@ars.usda.gov

See next page for additional authors

Follow this and additional works at: http://digitalcommons.unl.edu/hruskareports

Authors

Norasak Kalchayanand, Terrance M. Arthur, Joseph M. Bosilevac, John W. Schmidt, Rong Wang, Steven D. Shackelford, and Tommy L. Wheeler

Evaluation of Commonly Used Antimicrobial Interventions for Fresh Beef Inoculated with Shiga Toxin–Producing *Escherichia coli* Serotypes O26, O45, O103, O111, O121, O145, and O157:H7[†]

NORASAK KALCHAYANAND,* TERRANCE M. ARTHUR, JOSEPH M. BOSILEVAC, JOHN W. SCHMIDT, RONG WANG,
STEVEN D. SHACKELFORD, AND TOMMY L. WHEELER

*U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center,
Nebraska 68933-0166, USA*

ABSTRACT

Although numerous antimicrobial interventions targeting *Escherichia coli* O157:H7 have been developed and implemented to decontaminate meat and meat products during the harvesting process, the information on efficacy of these interventions against the so-called Big Six non-O157 Shiga toxin–producing *E. coli* (STEC) strains is limited. Prerigor beef flanks (160) were inoculated to determine if antimicrobial interventions currently used by the meat industry have a similar effect in reducing non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 compared with *E. coli* O157:H7. A high (10^4 CFU/cm^2) or a low (10^1 CFU/cm^2) inoculation of two cocktail mixtures was applied to surfaces of fresh beef. Cocktail mixture 1 was composed of O26, O103, O111, O145, and O157, while cocktail mixture 2 was composed of O45, O121, and O157. The inoculated fresh beef flanks were subjected to spray treatments by the following four antimicrobial compounds: acidified sodium chlorite, peroxyacetic acid, lactic acid, and hot water. High-level inoculation samples were enumerated for the remaining bacteria populations after each treatment and compared with the untreated controls, while low-level inoculation samples were chilled for 48 h at 4°C before enrichment, immunomagnetic separation, and isolation. Spray treatments with hot water were the most effective, resulting in mean pathogen reductions of 3.2 to 4.2 log CFU/cm^2, followed by lactic acid. Hot water and lactic acid also were the most effective interventions with the low-level inoculation on surfaces of fresh beef flanks after chilling. Peroxyacetic acid had an intermediate effect, while acidified sodium chlorite was the least effective in reducing STEC levels immediately after treatment. Results indicate that the reduction of non-O157 STEC by antimicrobial interventions on fresh beef surfaces were at least as great as for *E. coli* O157:H7. However, the recovery of these low inoculation levels of pathogens indicated that there is no single intervention to eliminate them.

Foodborne diseases caused by microorganisms are the number one food safety concern among consumers and regulatory agencies. Illnesses attributed to foodborne microorganisms can cause severe debilitating symptoms and in some cases, these illnesses can result in death. *Escherichia coli* O157:H7 is a common human infectious agent globally *(3),* and an estimated 63,153 *(24)* cases of *E. coli* O157:H7 infection occur in the United States annually. There are other serotypes of Shiga toxin–producing *E. coli* (STEC) called non-O157 STEC, which cause human disease similar to that produced by *E. coli* O157:H7. The Centers for Disease Control and Prevention (CDC) estimated that non-O157 STEC are responsible for about 112,752 cases of illness annually *(24)*. More than 200 virulent non-O157 serotypes have been isolated from outbreaks, sporadic cases

of hemolytic uremic syndrome, and severe diarrhea in the United States and other countries *(11)*.

In the United States, six O groups (comprising 13 serotypes) have been described by the CDC to be the cause of 71% of non-O157 STEC disease *(11)*. These serotypes have been identified as O26:H11 or nonmotile (NM); O45:H2 or NM; O103:H2, H11, H25, or NM; O111:H8 or NM; O121:H19 or H7; and O145:NM. The true number of illnesses caused by non-O157 STEC could be underestimated because detection and isolation of non-O157 STEC in stool and foodstuffs is laborious and time-consuming, with only about 4% of clinical laboratories routinely screening for these pathogens.

Previous studies have shown that beef cattle hides and feces carried non-O157 STEC at a prevalence of 4.6 and 55.9%, representing a potential source of beef carcass contamination *(17)*. Barkocy-Gallagher et al. *(6)* reported that the prevalence (56.6%) of non-O157 STEC on cattle hides is about the same as the prevalence of *E. coli* O157:H7 (60.6%). The prevalence (8%) of non-O157 STEC was reported on carcasses after the application of multiple hurdle

interventions *(5)*. Bosilevac et al. *(7)* reported that domestic boneless beef trim used for ground beef in the United States was contaminated with non-O157 STEC at a prevalence of 30%, while prevalence in U.S. commercial ground beef was 24.3% *(8)*.

Clearly, non-O157 STEC threaten consumers' health as well as cause economic loss because of illnesses, medical costs, or productivity losses. Furthermore, the impending implementation of U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) regulations to consider non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 as adulterants in certain raw beef products the same as for *E. coli* O157:H7 creates the need for approaches to control these top six non-O157 STEC. Although numerous interventions targeting *E. coli* O157:H7 have been developed and implemented to decontaminate meat and meat products during the harvesting process, the information on efficacy of these interventions against non-O157 STEC strains is limited. This study was designed to determine the effectiveness of existing antimicrobial interventions currently used in the meat industry for inactivating non-O157 STEC on fresh beef as compared with their effectiveness against *E. coli* O157:H7.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation of inocula. Strains of non-O157 *E. coli* serotypes O26 (:H11, 3891 and :H11, 3392, both human isolates), O45:H2 (01E-1269, human isolate), O45 WDG3 (isolated from cattle hide), O103 (:H2, 2421, human isolate), O111 (:NM, 1665, human isolate and :NM, ECRC 3007:85), O121:H19 (O1E-2074, human isolate), O121:H7 (isolated from ground beef), and O145 (:NM, GS5578620 and a ground beef isolate), and *E. coli* O157:H7 (ATCC 43895 and FSIS no. 4) from the U.S. Meat Animal Research Center (USMARC) culture collection were grown for 16 to 18 h at 37°C in nutrient broth (BD, Sparks, MD). Each strain was adjusted with 0.1% peptone solution to a cell concentration of approximately 1.5×10^8 CFU/ml, with a spectrophotometer value of 600 nm. Two cocktail mixtures were used in this study because there were no commercial immunomagnetic beads available for serogroups O45 and O121, making them difficult to separate from the other STEC strains during enumeration and detection. Cocktail mixture 1 consisted of an equal volume of each strain of O26, O103, O111, O145, and O157 to form a nine-strain cocktail mixture. Cocktail mixture 2 consisted of an equal volume of each strain of O45, O121, and O157 to form a six-strain mixture. These two cocktails were each diluted to approximately 1.5×10^7 or 1.5×10^4 CFU/ml for high and low inoculations, respectively. Cocktail mixture 1 was diluted in maximum recovery diluent, while cocktail mixture 2 was diluted with purge to provide a typical background flora. Purge was aseptically collected from vacuum-packaged beef subprimals that had been stored at −20°C and then thawed at 4°C. The inocula were then placed in an ice bath while processing each day's samples to restrict further cell growth before use.

Sample collection and inoculation. Prerigor beef flanks (cutaneous trunci muscle, 16 flanks for each treatment) were collected from a local beef cattle processing plant (80 flanks for mixture 1 and 80 for mixture 2) within 25 min postexsanguination and transported to the USMARC laboratory within 2 h in insulated containers. One intervention treatment per cocktail mixture combination was processed per day. Each day 16 flanks were divided into two groups of 8 flanks. The first group was inoculated with high levels of inocula, while the second group was inoculated with low levels of inocula. Before inoculation, each flank was divided into 16 25-cm² sections by using a template (10 by 10 cm), sterile cotton swab, and edible ink. An aliquot of 50 µl of either 1.5×10^7 (high) or 1.5×10^4 (low) CFU/ml of either cocktail mixture 1 or cocktail mixture 2 was inoculated on individual 25-cm² sections, spread over the area with a sterile cell spreader, and let stand undisturbed for 15 min at room temperature to allow bacterial cell attachment before subjecting the flanks to antimicrobial treatments. The final cell concentrations on meat surfaces for low and high inoculation were approximately 5×10^1 and 5×10^4 CFU/cm², respectively.

Intervention treatments. The antimicrobial compounds that were used in this study are generally regarded as safe approved, and the applied concentrations were within the recommended range. All the antimicrobial compounds were prepared according to the manufacturers' recommendations. The following four antimicrobial treatments were applied to the inoculated fresh beef flank tissue for 15 s: (1) acidified sodium chlorite (1,000 ppm; Ecolab, St. Paul, MN), (2) peroxyacetic acid (200 ppm; Ecolab), (3) lactic acid (4%; Purac, Chicago, IL), and (4) hot water (85°C) with a model spray wash cabinet with three oscillating spray nozzles (SS5010, Spray Systems Co., Wheaton, IL) at 60 cycles per min. Hot water (85°C at nozzles) was sprayed at 15 lb/in², while the other antimicrobial compounds were freshly prepared with water (22 to 25°C), sprayed at 20 lb/in² and dripped for 30 s. The distance between the spray nozzles and the beef flank tissue was 17 cm.

For the first set before subjecting each beef flank to antimicrobial treatment, four 25-cm² tissue sections were randomly excised and placed individually into four filtered bags (Whirl-Pak, Nasco, Ft. Atkinson, WI) to serve as controls. After treatments, another four 25-cm² tissue sections were excised and individually placed in another four filtered bags. For the second set, eight bags (four untreated control and four treated tissue samples) were stored for 48 h at 2 to 4°C before enumeration to determine residual effect on antimicrobial treatments. The first set of bags was enumerated within 10 min after the treatments.

Enumeration and culture. After 10 min posttreatment, untreated control and treated tissue samples (25-cm² section) were neutralized by adding 50-ml of Dey-Engley broth (BD, Sparks, MD) supplemented with 0.3% soytone, 0.25% sodium chloride, and homogenized for 1 min with a stomacher (BagMixer 400, Interscience, Weymouth, MA). For one set of high inoculation samples, a 1-ml aliquot of each sample was transferred into a 2-ml cluster tube and serially 10-fold diluted with maximum recovery diluents (BD). Appropriate dilutions were spiral plated on differential USMARC chromogenic agar plates and were enumerated on nonselective medium for aerobic plate count (APC) by using Petrifilm (3M, St. Paul, MN). The chromogenic medium was prepared as follows: Bacto Peptone (BD), 17.0 g/liter; Proteose Peptone (BD), 3.0 g/liter; sodium chloride (Sigma, St. Louis, MO), 5.0 g/liter; crystal violet (Sigma), 1.0 mg/liter; sorbose (Sigma), 6.0 g/liter; raffinose (Sigma), 6.0 g/liter; phenol red (Sigma), 20 mg/liter; bromothymol blue (Sigma), 1.5 mg/liter; and Bacto agar (BD), 15 g/liter. The medium pH was adjusted to 7.4 ± 0.1 and autoclaved for 10 min at 115°C. The medium was cooled to 50°C before adding filter-sterilized bile salts no. 3 (BD), 3 g/liter; 5-bromo-4-chloro-3-indoxyl-β-D-galactopyranoside (Gold Biotechnology, St. Louis, MO), 0.05 g/liter; isopropyl-β-D-thiogalactopyranoside (Sigma), 0.05 g/liter; novobiocin (Sigma), 5 mg/liter; and

potassium tellurite (Sigma), 0.125 mg/liter. The chromogenic plates were incubated at 37°C for 24 h and then at room temperature (~25°C) for 30 min for full color development for enumeration, while Petrifilms were incubated according to manufacturer's recommendation. Colony colors on the chromogenic medium were turquoise blue, blue-green, light green, dark blue green, light blue gray, purple, and green for O26, O45, O103, O111, O121, O145, and O157, respectively. The limit of detection (using a spiral plater; Spiral Biotech, Norwood, MA) was 60 CFU/cm^2.

After storage at 2 to 4°C for 48 h, the second set of high-inoculation tissue samples were enumerated as described above. CFUs were counted from Petrifilms and USMARC chromogenic agar plates compared with untreated controls. Colonies representing each STEC serogroup were counted and up to 20 presumptive colonies of combined plates of each of O26, O103, O111, O145, and E. coli O157:H7 were confirmed with multiplex PCR (13, 14, 21). For low-level inoculation, both control and treated samples were enriched at 25°C for 2 h, 42°C for 6 h, and held at 4°C before immunomagnetic separation (IMS) of target organisms.

A 1-ml aliquot of each enriched sample inoculated with cocktail mixture 1 was added to 25-μl mixtures of anti-O26, -O103, -O111, -O145, and -O157 immunomagnetic beads (Dynabeads, Invitrogen, Carlsbad, CA), and each enriched sample inoculated with cocktail mixture 2 was added to 20-μl of anti-O157 immunomagnetic beads (Dynabeads). Each enriched sample containing immunomagnetic beads was subjected to IMS as described previously (6, 20). The bacterial bead complexes were spread plated on CHROMAgar O15 (DRG International, Mountainside, NJ) supplemented with 5 mg of novobiocin per liter and 1.0 mg of potassium tellurite per liter for E. coli O157:H7, and on USMARC chromogenic agar for non-O157 STEC strains. The plates were incubated at 37°C for 22 to 24 h and then at room temperature for 30 min. Two presumptive colonies that have color characteristics for each serogroup were picked for confirmation with multiplex PCR (21).

Because there are no commercial immunomagnetic beads for serogroups O45 and O121, a 20-μl aliquot from each enrichment after IMS was streaked for isolation for O45 and O121 on USMARC chromogenic agar plates. The plates were incubated at 37°C for 22 to 24 h and then at room temperature for 30 min. Two presumptive colonies were picked for confirmation with multiplex PCR.

Statistical analyses. Colony counts were transformed to values expressed as log CFU per square centimeter from eight experimental replications of each treatment × four tissue sections ($n = 32$). One-way statistical analysis (analysis of variance) was performed with the general linear model procedure of SAS (SAS Institute Inc., Cary, NC). Least-squares means were calculated, and pairwise comparisons of means were determined with the Tukey-Kramer test method, with the probability level at $P \leq 0.05$. Percent recoveries for each organism were calculated from numbers of positive treated samples divided by numbers of positive untreated samples × 100 and were compared among organisms within treatments with WINPEPI Compare2 (1).

RESULTS AND DISCUSSION

Many countries such as the United States have implemented intervention-based hazard analysis and critical control points, where a specific procedure is applied to the product during processing in order to reduce the likelihood of pathogenic microorganisms being present on the carcasses and subsequent meat products. E. coli O157:H7 is the main target organism in contemporary fresh beef

production. The systems that provide reductions of 1 to 2 log units would be considered to provide appropriate improvements in the microbiological status of the product (4). In the present study, it is difficult to compare the non-O157 STEC results with the previous studies because of lack of methodological detail in those studies. Most of the antimicrobial interventions used in the beef industry are focused on reduction or elimination of E. coli O157:H7. Therefore, in the present study the effects of interventions on non-O157 STEC were compared with their effects against E. coli O157:H7, which was designed as the internal control.

High inoculation. There is little information indicating that currently used interventions are effective in reduction or inactivation of non-O157 STEC. High levels of organisms (approximately 10^4 CFU/cm^2) were inoculated in order to quantify the reduction in organisms from each treatment. In general, spray treatment with peroxyacetic acid, lactic acid, and hot water immediately reduced ($P < 0.05$) levels of serogroups O26, O45, O103, O111, O121, O145, O157, and APC compared with untreated controls (Table 1). The degree of inactivation depended on antimicrobial compound used and bacterial strains. Hot water and lactic acid effectively reduced all STEC strains, while peroxyacetic acid significantly reduced all STEC strains except O111, and acidified sodium chlorite did not significantly reduce serogroups O26, O111, and O145. Spray treatments with acidified sodium chlorite and storage at 4°C for 48 h resulted in additional reduction, which demonstrated this compound could be a long-acting microbial inhibitor and thus be suitable for prepackaged meat intervention. However, the other three treatments had little additional effect on pathogen levels after storage at 4°C.

Although acidified sodium chlorite, peroxyacetic acid, lactic acid, and hot water generally were able to reduce populations of STEC strains (Table 1), it was important to determine the level of reductions of non-O157 STEC compared with E. coli O157:H7. Although not always significantly different from the control, acidified sodium chlorite reduced STEC, ranging from 0.4 to 2.0 decimal reductions, with no difference ($P > 0.05$) between E. coli O157:H7 and non-O157 STEC in both mixtures (Table 2). Reductions of 1.9 to 2.3 log have been demonstrated in E. coli O157:H7 on beef carcass tissue by immersing into 500 ml of 0.02% acidified sodium chlorite activated with lactic acid for 30 s at ambient temperature (23).

Spray treatment with peroxyacetic acid at 200 ppm reduced ($P < 0.05$) the populations of STEC by 0.9 to 1.5 log, with no difference ($P > 0.05$) between the reduction of the non-O157 STEC and E. coli O157:H7 (except for O111). Similar results were reported when E. coli O157:H7–inoculated beef carcasses were treated with 200 ppm of peroxyacetic acid for 30 s (23). However, a marginal inactivation (0.7-log reduction) effect of peroxyacetic acid (123 lb/in^2 at 43°C for 15 s) on inoculated beef with E. coli O157:H7 was reported by King et al. (19).

Treatment with 4% lactic acid reduced ($P < 0.05$) non-O157 STEC by 1.6 to 3.1 log (Table 2). When compared

TABLE 1. *CFU counts after antimicrobial interventions in reducing STEC[a]*

| | Survivors after treatments (log CFU/cm^2) | | | | | | | | | |
| | Cocktail mixture 1 | | | | | | Cocktail mixture 2 | | | |
Treatment	O26	O103	O111	O145	O157	APC	O45	O121	O157	APC
Control	3.8 A[b]	4.0 A	3.5 A	4.1 A	3.8 A	5.4 A	4.5 A	3.9 A	4.5 A	5.4 A
ASC	3.1 A	3.0 B	2.8 A	3.5 A	3.4 A	4.1 B	2.6 B	1.9 B	2.6 B	3.7 B
ASC chilled	1.8 B	2.4 B	1.5 B	2.5 B	2.0 B	3.7 C	2.1 B	1.1 C	2.2 B	3.6 B
Control	4.3 A	2.9 A	3.0 A	4.3 A	4.2 A	5.2 A	4.4 A	4.1 A	4.4 A	5.4 A
POA	3.4 B	1.4 B	2.0 A	3.2 B	2.7 B	4.1 B	3.4 B	3.0 B	3.5 B	4.3 B
POA chilled	3.3 B	0.9 B	2.0 A	3.0 B	2.5 B	3.8 C	3.3 B	2.3 C	2.8 C	4.3 B
Control	3.7 A	3.6 A	3.7 A	4.5 A	2.5 A	5.3 A	4.2 A	4.3 A	4.2 A	5.2 A
LA	1.4 B	1.6 B	1.8 B	2.3 B	0.9 B	3.9 B	1.7 B	1.6 B	1.9 B	3.3 B
LA chilled	0.7 B	0.8 B	0.6 C	0.9 C	0.2 B	3.0 C	1.1 B	1.0 B	1.3 B	2.7 C
Control	4.2 A	4.0 A	4.2 A	4.5 A	4.3 A	5.3 A	4.0 A	3.6 A	4.6 A	5.6 A
HW	0.2 B	0.5 B	0.2 B	0.3 B	0.3 B	2.4 B	0.4 B	0.3 B	0.9 B	3.9 B
HW chilled	0.5 B	0.2 B	0.2 B	0.5 B	0.4 B	2.4 B	0.3 B	0.3 B	0.4 B	4.0 B

[a] APC, aerobic plate count; control, inoculated and sampled without any treatment; ASC, acidified sodium chlorite (1,000 ppm); POA, peroxyacetic acid (200 ppm); LA, lactic acid (4%); HW, hot water (85°C); chilled, samples were stored for 48 h at 4°C after treatment before enumeration. Each treatment, $n = 32$.
[b] Within a treatment type, means with no common letter that are in the same column of each inoculation study are significantly different ($P \leq 0.05$). Mean comparisons were performed independently for each cocktail mixture.

with *E. coli* O157:H7, there were no significant differences in the reduction of the non-O157 STEC strains. Similar results were reported from spray treatment with 2% lactic acid at 125 lb/in^2 for 15 s at 35°C where *E. coli* O111:H8 and *E. coli* O26:H11 were reduced to approximately the same extent as *E. coli* O157:H7 when these organisms were inoculated on fresh beef surfaces *(12)*. Similar reductions of *E. coli* O157:H7 also were reported when inoculated cheek meat was spray treated with 2% lactic acid for 26 s at 25 lb/in^2 and 25°C *(18)*. Ransom et al. *(23)* reported that lactic acid effectively reduced *E. coli* O157:H7 on inoculated beef carcass tissues. Lactic acid is most effective when applied at 50 to 55°C; however, the corrosive effect on the equipment seems to increase as the temperature rises *(2)*. Thus, the effects of lactic acid in the present study might have been greater if it had been applied at these higher temperatures.

Hot water treatment has been found to be effective against pathogens as well as spoilage bacteria *(9, 16, 18, 22)*. In the present study, the largest reductions of all STEC strains were observed when hot water was used as the antimicrobial intervention (Table 2). Hot water reduced ($P < 0.05$) the levels of the STEC strains studied, ranging from 3.2 to 4.2 log. Hot water reduced *E. coli* O157:H7 and the non-O157 STEC on beef surfaces equally, except for serogroup O103, which was the least sensitive to hot water treatment. Throughout the present study, some treated tissue samples were enumeration negative particularly from hot water and lactic acid treatments. These samples were subjected to an enrichment, IMS, and streak for isolation of target organisms. In almost all cases (95%), the target organisms were recovered from those samples (data not shown). This indicated that antimicrobial interventions either reduced levels below the enumeration detection limit or caused sublethal injury to the cells.

Antimicrobial treatments not only kill, but also inflict sublethal injury to microorganisms *(18)*. Therefore, using selective medium for enumeration may lead to overestimating the effectiveness of antimicrobial compounds, because

TABLE 2. *Compare inactivation efficiency of antimicrobial interventions between* Escherichia coli *O157:H7 and non-O157 STEC[a]*

| | Reduction of STEC (log CFU/cm^2) | | | | | | | | | |
| | Cocktail mixture 1 | | | | | | Cocktail mixture 2 | | | |
Treatment	O26	O103	O111	O145	O157	APC	O45	O121	O157	APC
ASC	0.7 A[b]	1.0 A	0.7 A	0.6 A	0.4 A	1.2 x	1.9 A	2.0 A	1.8 A	1.6 y
POA	1.1 A	1.5 A	1.0 A	1.1 A	1.5 A	1.1 x	0.9 A	1.1 A	0.9 A	1.0 x
LA	2.3 A	2.0 A	1.9 A	2.2 A	1.6 A	1.4 x	2.6 A	2.7 A	2.3 A	1.9 y
HW	4.0 A	3.6 B	4.0 A	4.2 A	4.0 A	2.9 y	3.6 A	3.2 A	3.8 A	2.4 z

[a] APC, aerobic plate count; ASC, acidified sodium chlorite (100 ppm); POA, peroxyacetic acid (200 ppm); LA, lactic acid (4%); HW, hot water (85°C). Each treatment, $n = 32$.
[b] Within a treatment type, means with no common capital letter (A through C) in the same row of each inoculation study are significantly different ($P \leq 0.05$). Among treatments, means with no common lowercase letter (x through z) in the same column of each inoculation study are significantly different ($P \leq 0.05$). Mean comparisons were performed independently for each cocktail mixture.

TABLE 3. *Recovery of STEC after antimicrobial interventions and chilled for 48 h at 4°C*

| | % recovery relative to untreated control[b] | | | | | | | |
| | Cocktail mixture 1 | | | | | Cocktail mixture 2 | | |
Treatment[a]	O26	O103	O111	O145	O157	O45	O121	O157
ASC	38 B[c]	44 B	47 AB	66 AB	69 A	54 A	38 A	61 AC
POA	78 A	78 A	62 A	78 A	70 A	54 A	56 A	75 A
LA	25 B	27 BC	17 B	35 B	13 B	18 B	21 A	19 B
HW	20 B	14 C	17 B	38 B	16 B	19 B	25 A	38 BC

[a] ASC, acidified sodium chlorite (1,000 ppm); POA, peroxyacetic acid (200 ppm); LA, lactic acid (4%); HW, hot water (85°C). Each treatment, $n = 32$. Dey-Engley broth was added to the bag containing tissue section for neutralization 10 min after treatment.

[b] Percent recoveries of each organism were calculated from numbers of positive treated samples divided by numbers of positive untreated control samples × 100. Untreated controls were inoculated and sampled without being subjected to any treatments and recovery rates ranged from 73 to 100%.

[c] Within a treatment type, recoveries with no common letter in the same row of each inoculation study are significantly different ($P \le 0.05$).

sublethally injured cells cannot propagate in the presence of selective agents. In the present study, both controls and treated samples were enumerated on a nonselective medium (aerobic count plate, 3M) to allow injured cells to resuscitate and multiply. The efficacy of antimicrobial compounds tested on APC is presented in Table 2. The inactivation ranged from a 1.1- to 2.9-log reduction for cocktail mixture 1, and ranged from a 1.0- to 2.4-log reduction in cocktail mixture 2. Antimicrobial interventions with lactic acid and hot water were more effective in reducing total bacteria counts than peroxyacetic acid and acidified sodium chlorite. Treatment with 200 ppm of peroxyacetic acid on chilled beef quarters resulted in little effect on total bacteria counts compared with 2 or 4% lactic acid *(15)*. The reduction of APC because of acidified sodium chlorite agreed with Bosilevac et al. *(10)* in that acidified sodium chlorite reduced APC by 1.0 to 1.5 log CFU in treated ground beef.

Low inoculation. The efficacy of acidified sodium chlorite, peroxyacetic acid, lactic acid, and hot water also was determined with a low level of organisms (10^1 CFU/cm^2), which could not be enumerated because of the detection limit. Both controls and treated samples were enriched and subjected to immunomagnetic separation before streaking for isolation. The recovery rates of treated samples after chilling for 48 h at 4°C were calculated and are presented in Table 3. The recovery of non-O157 STEC serotypes was similar or reduced relative to *E. coli* O157:H7 for all intervention treatments. The recovery rate of STEC serogroups O26, O45, O103, O111, O121, O145, and *E. coli* O157:H7 ranged from 13 to 78% and indicated that degree of killing and sublethal injury depends on type of antimicrobial intervention used. Thus, the recovery of these low inoculation levels of pathogens indicate that there is no single intervention to effectively eliminate them. The methodology used in lieu of commercially available anti-STEC O45 and O121 immunomagnetic beads could have resulted in underestimated recovery of O45 and O121.

Despite the reductions from these antimicrobial compounds in the levels of pathogens at high level inoculations, the recovery of these pathogens with low inoculation levels indicated that one of the following possibilities could occur: (i) the solutions might not be applied uniformly to all of the surfaces as carcasses have irregular shapes and surfaces causing overexposure to the treatment on one part and underexposure on others; or (ii) even with a uniform spray, all antimicrobial compounds will inactivate some bacterial cells and inflict sublethal injury to other cells. An enumeration with selective medium might overestimate effects of the antimicrobial interventions used because in a suitable environment, sublethally injured cells could repair their injuries, gain their normal characteristics, and subsequently initiate multiplication.

This study was conducted to determine whether antimicrobial compounds currently used by the meat industry are effective against non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 compared with *E. coli* O157:H7. Based on these findings, antimicrobial compounds commonly used by the beef industry were effective against the top six non-O157 STEC to varying degrees. In general, the compounds tested were as effective against non-O157 STEC as *E. coli* O157:H7. The degree of effectiveness depended on the antimicrobial compounds used and the organism. Hot water was the most effective in reducing non-O157 STEC followed by lactic acid, while acidified sodium chlorite was the least effective.

REFERENCES

1. Abramson, J. H. 2011. WINPEPI updated: computer programs for epidemiologists and their teaching potential. *Epidemiol. Perspect. Innov.* 8:1.
2. Acuff, G. R. 2005. Chemical decontamination strategies for meat, p. 351–353. *In* J. N. Sofos (ed.), Improving the safety of fresh meat. Woodhead Publishing, Ltd., New York.

3. Anonymous, National Institute of Health and Infectious Disease. 1997.Verocytotoxin-producing *Escherichia coli* (enterohemorrhagic *E. coli*) infections, Japan 1996–June 1997. *Infect. Agents Surveill. Rep.* 18:153–154.

4. Anonymous. 2003. Pathogen reduction interventions for carcasses. Meat technology update newsletter 03/2, April 2003. Available at: http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_ UPDATE_03-2.pdf. Accessed 14 February 2012.

5. Arthur, T. M., G. A. Barkocy-Gallagher, M. River-Betancourt, and M. Koohmaraie. 2002. Prevalence and characterization of non-O157 Shiga toxin–producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* 68:4847–4852.

6. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.

7. Bosilevac, J. M., M. N. Guerini, D. M. Brichta-Harhay, T. M. Arthur, and M. Koohmaraie. 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. *J. Food Prot.* 70:440–449.

8. Bosilevac, J. M., and M. Koohmaraie. 2011. Prevalence and characterization of non-O157 Shiga toxin–producing *Escherichia coli* isolates from commercial ground beef in the United States. *Appl. Environ. Microbiol.* 77:2103–2112.

9. Bosilevac, J. M., X. Nou, G. A. Barkocy-Gallagher, T. M. Arthur, and M. Koohmaraie. 2006. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of *Escherichia coli* O157:H7 on preevisceration beef carcasses. *J. Food Prot.* 69:1808–1813.

10. Bosilevac, J. M., S. D. Shackelford, R. Fahle, T. Biela, and M. Koohmaraie. 2004. Decreased dosage of acidified sodium chlorite reduces microbial contamination and maintains organoleptic qualities of ground beef products. *J. Food Prot.* 67:2248–2254.

11. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 Shiga toxin–producing *Escherichia coli* infections in the United States, 1983–2002. *J. Infect. Dis.* 192:1422–1429.

12. Cutter, C. N., and M. Rivera-Betancourt. 2000. Interventions for the reduction of *Salmonella* Typhimurium DT 104 and non-O157:H7 enterohemorrhagic *Escherichia coli* on beef surfaces. *J. Food Prot.* 63:1326–1332.

13. DebRoy, C., P. M. Fratamico, E. Roberts, M. A. Davis, and Y. Liu. 2005. Development of PCR assays targeting genes in O-antigen gene clusters for detection and identification of *Escherichia coli* O45 and O55 serogroups. *Appl. Environ. Microbiol.* 71:4919–4924.

14. Fratamico, P. M., C. E. Briggs, D. Needle, C. Chen, and C. DebRoy. 2003. Sequence of the *Escherichia coli* O121 O-Antigen gene cluster and detection of enterohemorrhagic *E. coli* O121 by PCR amplification of the *wzx* and *wzy* genes. *J. Clin. Microbiol.* 41:3379–3383.

15. Gill, C. O., and M. Badoni. 2004. Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. *Int. J. Food Microbiol.* 91:43–50.

16. Gill, C. O., J. Bryant, and D. Bedard. 1999. The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcasses sides *Food Microbiol.* 16:281–289.

17. Hussein, H. S. 2007. Prevalence and pathogenicity of Shiga toxin–producing *Escherichia coli* in beef cattle and their products. *J. Anim. Sci.* 85:63–72.

18. Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, T. L. Wheeler, and M. Koohmaraie. 2008. Evaluation of various antimicrobial interventions for the reduction of *Escherichia coli* O157:H7 on bovine heads during processing. *J. Food Prot.* 71:621–624.

19. King, D. A., L. M. Lucia, A. Castillo, G. R. Acuff, K. B. Harris, J. W. Savell. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. *Meat Sci.* 69:401–407.

20. Nou, X., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2006. Improvement of immunomagnetic separation for *Escherichia coli* O157:H7 detection by the PickPen magnetic particle separation device. *J. Food Prot.* 69:2870–2874.

21. Perelle, S., F. Dilasser, J. Grout, and O. Fach. 2004. Detection by 5′-nuclease PCR of Shiga toxin–producing *Escherichia coli* O26, O55, 91, O103, O111, O103, O145, and O157:H7, associated with the world's most frequent clinical cases. *Mol. Cell. Probes* 18:185–192.

22. Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1977. Comparison of steam pasteurization and other methods for reduction of beef carcasses at various locations in processing. *J. Food Prot.* 60:476–484.

23. Ransom, J. R., K. E. Belk, J. N. Sofos, J. D. Stopforth, J. A. Scanga, and G. C. Smith. 2003. Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 on beef cuts and trimmings. *Food Prot. Trends* 23:24–34.

24. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.