Evaluation of the efficacy of commonly used antimicrobial interventions on Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145, and O157

Final report

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Objectives: The overall objective was to determine effectiveness of existing antimicrobial compound treatments currently used in meat industry on inactivation of STEC inoculated fresh beef.

Conclusions: A study was conducted to determine whether antimicrobial compounds currently used by the meat industry are effective against non-O157 STEC serogroups O26, O103, O111 and O145 compared to *E. coli* O157:H7. Six antimicrobial compounds used were acidified sodium hypochlorite, peroxyacetic acid, FreshFx, lactic acid, activated hydrobromic acid, and hot water. Based on these findings, all antimicrobial compounds used by meat industry appear to be effect against non-O157 STEC and the reductions of these pathogens on inoculated fresh beef were equally effective against *E. coli* O157:H7. The degree of effectiveness depended on the antimicrobial compounds used. In the present study, hot water was the most effective in reducing pathogens tested followed by lactic acid. Acidified sodium chlorite and activated hydrobromic acid showed less immediate effect, but increased effectiveness after chilling for 48 h at 4^{o}C.

Deliverable: The results will assist the meat industry by identifying antimicrobial compounds suitable where processing steps need to be applied for controlling these pathogens, and, thus, enhance meat safety.

Technical Abstract

Ninety six pre-rigor beef flanks were used to conduct a study in order to determine if antimicrobial interventions currently used by the meat industry have an effect in reducing non-O157 STEC serogroups O26, O103, O111, O145, and *E. coli* O157:H7 inoculated fresh beef. Two inoculation levels, high (10^4 CFU/cm^2) and low (10^1 CFU/cm^2), of a nine-strain cocktail mixture were inoculated on surfaces of fresh beef and subjected to the following six antimicrobial interventions: acidified sodium chlorite (1000 ppm), peroxyacetic acid (200 ppm), FreshFx (1:50), lactic acid (4%), activated hydrobromic acid (300 ppm), and hot water (85^{o}C). High levels of inoculation samples were enumerated for the remaining bacteria populations following each treatment, while low levels of inoculation samples were chilled for 48 h at 4^{o}C before enrichment, immunomagnetic separation, and isolation. Of the antimicrobial interventions studied, spray treatments with hot water were the most effective, resulting in pathogen reductions of ≥ 3.5 log CFU/cm^2, followed by 4% lactic acid (≥ 1.6 log CFU/cm^2). Similar effectiveness of hot water and lactic acid also were found with low levels of inoculation on surface of beef flanks. Both FreshFx and peroxyacetic acid had an intermediate effect in reducing pathogens studied. Acidified sodium chlorite and activated hydrobromic acid were the least effect in reducing pathogens, but the effectiveness increased after chilling for 48 h at 4^{o}C. Results indicated that antimicrobial interventions used to reduce *E. coli* O157:H7 on fresh beef surfaces were equally effective against non-O157 STEC O26, O103, O111, and O145.

Introduction

Although *E. coli* O157:H7 is currently most widely recognized, more than 100 other non-O157 Shiga toxin producing *E. coli* (STEC) serotypes have been implicated in cases of human disease. Non-O157 STEC strains are classified as emerging pathogens, often of bovine origin (Hussein, 2007; Hussein and Sakuma, 2005). These organisms cause severe disease the same as *E. coli* O157:H7 does including hemolytic uremic syndrome. Currently, FSIS is developing a testing program for non-O157 STEC from meat products associated with slaughter/dressing and further processing operations. The previous studies showed that the beef cattle going to slaughter carried non-O157 STEC between 2.1 and 70.1%, which represent a potential source of contamination (Hussein, 2007; Hussein and Sakuma, 2005). The contamination of beef products with non-O157 STECs are probably the same or similar to *E. coli* O157:H7. Barkocy-Gallagher et al (2003) reported that the prevalence of non-O157 STEC (56.6%) on cattle hides is nearly as high as the prevalence of *E. coli* O157:H7 (60.6%). The prevalence of non-O157 STECs in imported and domestic boneless beef trim used for ground beef in the U.S. has recently been reported as high as 10 to 30% (Bosilevac et al., 2007).

Worldwide non-O157 STEC outbreaks emerged in the 1980's, but the first reported outbreaks in the U.S. occurred in the 1990's (Hussein 2007; Brooks et al., 2005). Seven non-O157 STEC outbreaks were reported in the U.S. during 1983 to 2002 (Brook et al., 2005). CDC documented an additional five non-O157 STEC outbreaks from 2003 to 2007. CDC also estimates that non-O157 STEC are responsible for about 1,579 confirmed cases of illness annually (Scallan et al., 2011). The most common serotypes reported to cause foodborne illness in the U.S. are O26, O45, O103, O111, O121, and O145 (Brooks et al., 2005). Serogroup O26 was the only 1 non-O157 outbreak in the U.S. associated with meat (beef) and sickens 3 people in 2010. Clearly, non-O157 STEC strains are a threat to consumers' health as well as economy

loss due to illnesses from these pathogens. At this point, non-O157 STEC becomes an issue that FSIS may declare selected non-O157 STECs to be adulterants. 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 on non-O157 STECs is very limited.

Materials and Methods

Bacterial strains, growth conditions, and preparation of inoculum: Two strains each of non-O157 *E. coli* serotypes O26 (:H11, 3891 and :H11, 3392 both human isolates), O103 (:H2, 2421 human isolate), O111 (:NM, 1665 human isolate and :NM, ECRC 3007:85) and O145 (:NM, GS5578620 and a ground beef isolate) and *E. coli* O157:H7 (ATCC 43895 and FSIS #4) from USMARC culture collection were grown for 16 to 18 h at 37^{o}C in nutrient broth (Beckton Dickinson, Sparks, MD). Each strain was adjusted with 0.1% peptone solution to a cell concentration of approximately 1.5 x 10^8 CFU/ml using a spectrophotometer at 600 nm. An equal volume of each strain will be mixed to form a 9-strain cocktail mixture and will be diluted to 1.5 x 10^7 and 1.5 x 10^4 CFU/ml for high and low inoculation, respectively. The inoculums were placed in an ice-bath to prevent further cell growth during inoculation study.

Fresh beef inoculation: A total of ninety-six pre-rigor beef flanks (cutaneous trunci muscle; 16 flanks for each treatment) were collected from a local beef cattle processing plant and were used in this study. Each flank was divided into four 100-cm^2 sections and each 100-cm^2 was divided into four 25-cm^2 sections using template and edible ink. Two inoculation levels 10^1 (low) and 10^4 (high) CFU/cm^2 were inoculated on surfaces of marked flanks. An aliquot of 50 µl of either 1.5 x 10^7 or 1.5 x 10^4 CFU/ml of cocktail mixture was inoculated on individual 25-cm^2 sections, spread over the area, and let stand 20 min at room temperature to allow bacterial cells attachment before subjecting to antimicrobial treatments. The final cell concentrations for low and high inoculation were approximately 5 x 10^1 and 5 x 10^4 CFU/cm^2, respectively.

Antimicrobial treatments and sampling: The antimicrobial compounds that were used in this project are GRAS approved and the applied concentrations were within the recommended range. The following six antimicrobial treatments were applied to the inoculated fresh beef for 15 s: (1) acidified sodium chlorite (1000 ppm, pH = 2.3; Ecolab, MN), (2) peroxyacetic acid (200 ppm, pH = 2.8; Ecolab), (3) FreshFx (1:50, pH = 1.8; SteriFX Inc., Shreveport, LA), (4) lactic acid (4%, pH = 2.2; PURAC, IL), (5) activated hydrobromic acid (300 ppm, pH = 7.4; Enviro Tech, CA), and (6) Hot water (85^{o}C) using a model spray wash cabinet with three oscillating spray nozzles (SS5010; Spray Systems Co., Wheaton, IL) at 60 cycles per min. Hot water (85^{o}C at nozzles) was be sprayed at 15 psi, while the other antimicrobial compounds were freshly prepared with water (22 to 25^{o}C) and sprayed at 20 psi The distance between nozzles and beef flank was 17 cm. Before subjecting to antimicrobial treatments, four 25-cm^2-tissue sections (one from each 100-cm^2 of marked inoculated beef) were excised and placed individually into filtered bags (Whirl-Pak, Nasco, Ft. Atkinson, WI) to serve as controls. After treatments, another four 25-cm^2-tissue sections were excised and placed in filtered bags. One set of bags (control and treated tissue samples) was stored for 48 h at 2 to 4^{o}C before enumeration to determine residual effect on antimicrobial treatments. The other set of bags was enumerated within 10 min following the treatments.

Microbiological and statistical analyses: Control and treated tissue samples (25-cm^2 section) were neutralized by adding 50-ml of Dey/Engley broth (Beckton Dickinson) supplemented with 0.3% soytone and 0.25% sodium chloride and homogenized for 1 min at 540 rpm using a stomacher (BagMixer® 400; Interscience, Weymouth, MA). For the high inoculation samples, 1-ml aliquot of each sample was transferred into 2-ml cluster-tube and was serially10-fold diluted with maximum recovery diluents (Becton Dickinson). Appropriate dilutions were spiral plated on in house selective U.S. Meat Animal Research Center (USMARC) chromogenic medium and were plated on non-selective medium for aerobic plate count (APC) using petrifilms (3M, St. Paul, MN). The chromogenic plates were incubated at 37°C for 24 h and at room temperature for 30 min for full color development for enumeration and petrifilms were incubated according to manufacturer's recommendation. The limit of detection using a spiral plater (Spiral Biotech, Norwood, MA) was 60 CFU/cm^2. Following storage at 2 to 4°C for 48 h, the second set of high inoculation tissue samples were enumerated as described above. Colony-forming units were counted from petrifilms and USMARC chromogenic agar plates compared to untreated controls. Colony colors representing each STEC serogroup were counted and up to 20 presumptive colonies of combined plates of each of O26, O103, O111, O145, and O157 were picked for confirmation using multiplex PCR (Perelle et al., 2004). For low level inoculation, both controls and treated samples were enriched at 25°C for 2 h, 42°C for 6 h, and held at 4°C before immunomagnetic separation of target organisms. One milliliter aliquot of each enriched sample was added to 25-µl mixtures of anti-O26, -O103, -O111, -O145, and -O157 immunomagnetic beads (Dynabeads, Invitrogen, Carlsbad, CA) and subjected to immunomagnetic separation. The bead-bacteria complexes were spread plated on the USMARC chromogenic agar plates at 37°C for 22 to 24 h and at room temperature for 30 min. Two presumptive colonies that have color characteristics for each serogroup were picked for confirmation using multiplex PCR.

Colony counts were transformed to $\log_{10}$CFU/cm^2 values from eight experimental replications. One-way statistical analysis (Analysis of Variance, ANOVA) was performed using the general Linear Model procedure of SAS (SAS Institute, Inc., Cary, NC). Least squared means wer calculated and pairwise comparisons of means were determined using Tukey-Kramer test method with the probability level at $P \leq 0.05$.

Results and Discussion

It is difficult to compare the non-O157 STEC results of this study to the previous studies, because there was little information. Most of the antimicrobial interventions used in meat industry are focused on reduction or elimination of *E. coli* O157:H7. Therefore, in this study the results were compared to intervention against *E. coli* O157:H7. Two inoculation studies were conducted, high and low levels of inoculation.

High inoculation study: The current antimicrobial interventions used in meat industry are designed to reduce or inactivate *E. coli* O157:H7. However, there is little information that these interventions are effective in reduction or inactivation of non-O157 STEC. In this study, *E. coli* O157:H7 was included in a cocktail mixture of non-O157 STEC in order to compare the effectiveness of each antimicrobial treatment between non-O157 STEC and *E. coli* O157:H7. High levels of organisms (approximately 10^4 CFU/cm^2) were inoculated in order to be able to

demonstrate the effectiveness of each treatment. The effectiveness of acidified sodium chlorite, peroxyacetic acid, FreshFx, and lactic acid is presented in Table 1. Acidified sodium chlorite reduced ($P > 0.05$) non-O157 STEC O26, O111, O145, and *E. coli* O157:H7 ranging from 0.4 to 0.7 log reduction). Acidified sodium chlorite significantly reduced ($P < 0.05$) serogroup O103 (from 3.9 to 2.8 log CFU/cm^2) following spray treatment. Some studies have demonstrated a 1.9-2.3 log reduction in *Salmonella* and *E. coli* O157 on beef carcass tissue using a wash/spray of sodium chlorite activated (acidified) with citric acid (Ransom *et al.* 2003). Castillo et al. (1999) reported that up to 4.6 log reductions in *E. coli* O157:H7 and *Salmonella* when a water wash was used and followed by an acidified sodium chlorite spray. However, limited success using acidified sodium chlorite spray treatment was reported by Gill and Badoni (2004). The chilled samples after the spray treatment (48 h at 4^{o}C) reduced ($P < 0.05$) STEC O26, O111, O145, and *E. coli* O157:H7 from 3.1 to 1.8, 2.8 to 1.5, 3.5 to 1.5, and 3.4 to 2.0 log CFU/cm^2, respectively. The results of chilled samples after treatment suggested that acidified sodium chlorite may be a long-acting microbial inhibitor and may be suitable for pre-packaged meat.

Table 1. Effectiveness of acidic antimicrobial compounds in reducing non-O157 STEC and *E. coli* O157:H7.

	Survivors of STEC on selective medium[b] (log CFU/cm^2)				
Treatment[a]	O26	O103	O111	O145	O157
Control	3.8^A	4.0^A	3.5^A	4.1^A	3.8^A
ASC	3.1^A	3.0^B	2.8^A	3.5^A	3.4^A
$ASC_{chilled}$	1.8^B	2.4^B	1.5^B	2.5^B	2.0^B
Control	4.3^A	2.9^A	3.0^A	4.3^A	4.2^A
POA	3.4^B	1.4^B	2.0^A	3.2^B	2.7^B
$POA_{chilled}$	3.3^B	0.9^B	2.0^A	3.0^B	2.5^B
Control	4.4^A	3.9^A	3.7^A	4.6^A	4.4^A
Fx	2.7^B	2.8^B	3.0^A	3.4^B	3.0^B
$Fx_{chilled}$	1.9^C	1.9^C	1.9^B	2.6^C	1.6^C
Control	3.7^A	3.6^A	3.7^A	4.5^A	2.5^A
LA	1.4^B	1.6^B	1.8^B	2.3^B	0.9^B
$LA_{chilled}$	0.7^B	0.8^B	0.6^C	0.9^C	0.2^B

[a] Control, inoculated and sampled without any treatment; ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid. Chilled, samples were stored for 48 h at 4^{o}C following treatment before enumeration. Each treatment, n = 32.
[b] USMARC chromogenic medium.
Within a treatment type, means with no common letter that are in the same column are significantly different ($P \leq 0.05$).

Spray treatment with peroxyacetic caid at 200 ppm immediately reduced ($P < 0.05$) the population of O26, O103, O145, and *E. coli* O157:H7, except O111 on surface of beef flanks

(4.3 to 3.4, 2.9 to 1.4, 4.3 to 3.2, 4.2 to 2.7, and 3.0 to 2.0 log CFU/cm^2, respectively). Similar results of spray treatment with peroxyacetic acid on *E. coli* O157:H7 inoculated beef carcasses has been reported (Ransom et al., 2003). However, marginal inactivation (0.7 log reduction) effect of peroxyacetic acid on inoculated beef with *E. coli* O157:H7 and *Salmonella* was reported (King et al., 2005). Chilled samples after peroxyacetic acid treatment did not have an additional reduction ($P > 0.05$) compared to samples immediately treated.

FreshFx reduced ($P < 0.05$) the population of STEC O26, O103, O145, and *E. coli* O157:H7 except serogroup O111 ($P > 0.05$) (4.4 to 2.7, 3.9 to 2.8, 4.6 to 3.4, 4.4 to 3.0, and 3.7 to 3.0 log CFU/cm^2, respectively). Similar reduction of *E. coli* O157:H7 was reported on inoculated beef head after spray treatment with FreshFx (Kalchayanand et al., 2008). Additional reduction was found on all STEC tested after chilling for 48 h at 4^{o}C following tratment with FreshFx.

Treatment with 4% lactic acid reduced ($P < 0.05$) non-O157 STEC serogroups O26, O103, O111, O145, and *E. coli* O157 from 3.7 to 1.4, 3.6 to 1.6, 3.7 to 1.8, 4.5 to 2.3, and 2.5 to 0.9 log CFU/cm^2, respectively. Ransom et al. (2003) reported that lactic acid effectively reduced *E. coli* O157:H7 inoculated beef carcass tissues. Lactic acid is most effective when applied at 50 to 55^{o}C; however, the corrosive effect on the equipment seems to increase as the temperature rises (Acuff, 2005). Additional reductions ($P < 0.05$) were on serogroup O111 and O145 after chilling 48 h at 4^{o}C following lactic acid treatment.

The efficacy of activated hydrobromic acid and hot water is presented in Table 2. Hydrobromic acid was activated with 12.5 sodium hypochlorite to from a compound called hypobromous acid (HOBr) as shown in equation (1), which is an active antimicrobial agent (Sun et al., 1995 and Panangala et al., 1997).

$$HBr + NaOCl = NaCl + HOBr \qquad (1)$$

In previous study (Kalchayanand et al., 2008), HOBr was generated from a compound called 1, 3- Dibromo-5, 5 dimethylhydantoin (DBDMH). This compound in water hydrolyses to HOBr as shown in equation (2).

$$DBDMH + H_2O = 2HOBr + DMH \qquad (2)$$

Activated hydrobromic acid (pH = 7.4) reduced ($P < 0.05$) STEC O26, O103, O111, O145, and *E. coli* O157:H7 on inoculated beef flanks from 4.4 to 3.6, 4.2 to 3.4, 4.3 to 3.6, 4.6 to 4.0, and 4.2 to 3.7 log CFU/cm^2, respectively. When activated hydrobromic acid treated samples were chilled for 48 h at 4^{o}C, the population of O26, O103, O111, O145, and *E. coli* O157:H7 were reduced ($P < 0.05$) from 3.6 to 2.4, 3.4 to 2.3, 3.6 to 2.9, 4.0 to 3.2, and 3.7 to 30 log CFU/cm^2, respectively. When HOBr was prepared using DBDMH, the reduction of inoculated *E. coli* O157:H7 was higher than HOBr prepared from activated hydrobromic acid. The authors are investigating to determine this discrepancy.

The reductions of all STEC O26, O103, O111, O145, and *E. coli* O157:H7 also were observed when hot water was used as antimicrobial intervention. Hot water reduced ($P < 0.05$) aforementioned STEC from 4.2 to 0.2, 4.0 to 0.5, 4.2 to 0.2, 4.5 to 0.5, and 4.3 to 0.3 log

CFU/cm^2, respectively. There was no additional reduction ($P > 0.05$) of all STEC tested when hot water treated samples were chilled for 48 h at 4^{o}C. Hot water treatment has been found to be

Table 2. Effectiveness of neutral antimicrobial compounds in reducing non-O157 STEC and *E. coli* O157:H7.

Treatment[a]	Survivors of STEC on selective medium[b] (log CFU/cm^2)				
	O26	O103	O111	O145	O157
Control	4.4^A	4.2^A	4.3^A	4.6^A	4.2^A
AHBr	3.6^B	3.4^B	3.6^B	4.0^B	3.7^B
$AHBr_{chilled}$	2.4^C	2.3^C	2.9^C	3.2^C	3.0^C
Control	4.2^A	4.0^A	4.2^A	4.5^A	4.3^A
HW	0.2^B	0.5^B	0.2^A	0.3^B	0.3^B
$HW_{chilled}$	0.5^B	0.2^B	0.2^A	0.5^B	0.4^B

[a] Control, inoculated and sampled without any treatment; AHBr, activated hydrobromic acid; HW, hot water. Chilled, samples were stored for 48 h at 4^{o}C following treatment before enumeration. Each treatment, n = 32.
[b] USMARC chromogenic medium.
Within a treatment type, means with no common letter that are in the same column are significantly different ($P \leq 0.05$).

effective against pathogens as well as spoilage bacteria (Bosilevac et al., 2006, Phebus et al., 1997; Gill et al., 1999; Kalchayanand et al., 2008 and 2009).

Although acidified sodium chlorite, peroxyacetic acid, FreshFx, lactic acid, activated hydrobromic acid, and hot water were generally able to reduce population of STEC (Table 1 and 2), it is important to determine which antimicrobial compounds effectively reduced non-O157 STEC compared to *E. coli* O157:H7. Reduction of STEC population on inoculated fresh beef flanks due to antimicrobial compounds used is presented in Table 3. Acidified sodium chloride reduced STEC ranging from 0.4 to 1.0 log reduction, which showed no significant difference ($P > 0.05$) between *E. coli* O157:H7 and non-O157 STEC. Similar results were observed when peroxyacetic acid was used as antimicrobial agent. Peroxyacetic acid reduced STEC ranged from 1.0 to 1.5 log reduction. Although peroxyacetic acid had less effective in reducing serogroups O26, O111, and O145, these pathogens did not differ ($P > 0.05$) from serogroup O103 and *E. coli* O157:H7. FreshFx significantly reduced ($P < 0.05$) serotype O26 compared to serotype O103, O111, and O145, but did not differ ($P > 0.05$) from *E. coli* O157:H7. There was no significant difference between non-O157 STEC and *E. coli* O157:H7 when beef flanks were inoculated with these pathogens and spray treated with lactic acid. Lactic acid reduced STEC ranging from 1.6 to 2.3 log reduction, where *E. coli* O157:H7 was the least sensitive to lactic acid.

Activated hydrobromic acid reduced STEC ranging from 0.5 to 0.8 log reduction, which *E. coli* O157:H7 was the least sensitive to the treatment compared to non-O157 STEC serogroups O26, O103, O111, and O145 (Table 3). Hot water reduced STEC inoculated beef flanks ranging from

3.5 to 4.2 log reduction, which serogroup O103 was the least sensitive to hot water treatment compared to *E. coli* O157:H7 and serogroups O26, O111, and O145. There were no differences between *E. coli* O157:H7 and O26, O111, and O145 with hot water treatment (Table 3). Based on the enumeration on selective chromogenic medium, the order of inactivation of tested antimicrobial compounds is as follows: hot water > lactic acid > FreshFx ≥ peroxyacetic acid > acidified sodium chlorite = activated hydrobromic acid. Both acidified sodium chlorite and activated hydrobromic acid had less immediate effect in reducing non-O157 STEC and *E.coli* O157:H7, but increased effectiveness in inactivation after chilling for 48 h at 4°C.

Table 3. Compare inactivation efficiency of antimicrobial compounds between *E. coli* O157:H7 and non-O157 STEC.

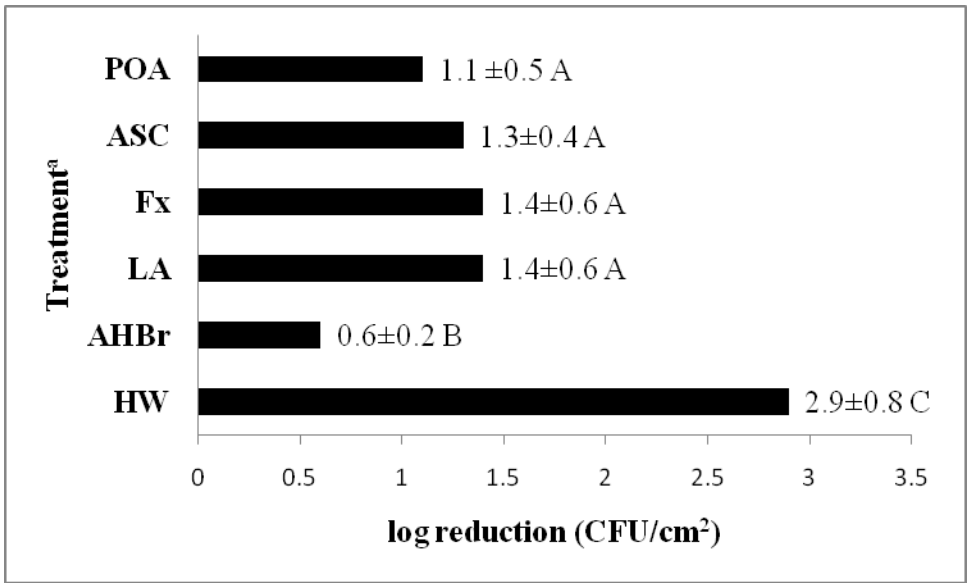
	Reduction of STEC on selective medium[b] (log CFU/cm^2)				
Treatment[a]	O26	O103	O111	O145	O157
ASC	0.7^{A}	1.0^{A}	0.7^{A}	0.6^{A}	0.4^{A}
POA	1.2^{A}	1.5^{A}	1.0^{A}	1.1^{A}	1.5^{A}
Fx	1.7^{B}	1.1^{A}	0.7^{A}	1.2^{A}	1.4^{AB}
LA	2.3^{A}	2.0^{A}	1.9^{A}	2.2^{A}	1.6^{A}
AHBr	0.8^{A}	0.8^{A}	0.7^{A}	0.6^{A}	0.5^{B}
HW	4.0^{A}	3.5^{B}	4.0^{A}	4.2^{A}	4.0^{A}

[a] Control, inoculated and sampled without any treatment; ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; AHBr, activated hydrobromic acid; HW, hot water. Each treatment, n = 32.
[b] USMARC chromogenic medium.
Within a treatment type, means with no common letter that are in the same row are significantly different ($P \leq 0.05$).

Antimicrobial treatments not only inactivate but also inflict sublethal injury to microorganisms. Therefore, using selective medium to enumerate may lead to overestimate the effectiveness of antimicrobial compounds because sublethally injured cells cannot propagate in the presence of selective agents. In this study, both controls and treated samples also were enumerated on non-selective medium. Non-selective medium (aerobic count plate, 3M) allows sublethally injured cells to resuscitate and grow on this medium. The efficacy of antimicrobial compounds tested on aerobic plate counts (APC) is presented in Figure 1. The inactivation ranged from 0.6 to 2.9 log reduction, which hot water was the most effective and activated hydrobromic acid was the least effective in reducing APC. Similar results of reduction of APC with hot water treatment, but not with bromine compound have been reported (Kalchayanand et al., 2009). Spray treatment of beef flanks (inoculated with fecal solution containing mixture of *E. coli* O157:H7 and *Salmonella*) with DBDMH at 35 psi for 12 s resulted in approximately 3 log reduction (Kalchayanand et al., 2009), which was contrast with spray treatment with hydrobromic acid even though the end

product of both chemicals is hypobromous acid (HOBr). Lactic acid, FreshFx, acidified sodium chlorite, and peroxyacetic acid had equal effect on reduction of APC. In a commercial trial, the effect of a solution of 200 ppm peroxyacetic acid on chilled beef quarters was investigated (Gill and Badoni, 2004). The results indicated that peroxyacetic acid treatment had little effect on total bacteria counts compared to 2 or 4% lactic acid. The reduction of APC due to acidified sodium chlorite agreed with Bosilevac et al (2004) that acidified sodium chlorite reduced APC by 1.0 to 1.5 log of treated ground beef.

Figure 1. Efficacy of antimicrobial compounds on the reduction of aerobic bacteria.



[a] POA, peroxyacetic acid; ASC, acidified sodium chlorite; Fx, FeshFx; LA, lactic acid; AHBr, activated hydrobromic acid; HW, hot water. Each treatment, n = 32.
A-C, Means bearing with no common letter are significantly different ($P \leq 0.05$).

Low inoculation study: The efficacy of acidified sodium chlorite, peroxyacetic acid, FreshFx, lactic acid, activated hydrobromic acid, and hot water also was determined with low levels of organisms (10^1 CFU/cm^2), which could not be enumerated due to detection limit. Both controls and treated samples were enriched and subjected to immunomagnetic separation before streaking for isolation. The recovery rates of controls and treated samples after chilling for 48 h at 4^{o}C were calculated and are presented in Table 4. The recovery rate of non-O157 and *E. coli* O157:H7 STEC ranged from13.3 to 96.9%. Hot water and lactic acid were the most effective in reducing *E. coli* O157:H7 and non-O157 STEC O26, O103, O111, and O145.

Table 4. Recovery of non-O157 STECs after intervention treatments and chilling for 48 h at 4°C.

	% recovery relative to untreated controls				
Treatment[a]	O26	O103	O111	O145	O157
POA	78.1	78.1	62.1	78.1	69.6
LA	25.0	26.7	17.4	35.0	13.3
Fx	93.8	53.8	43.8	78.1	96.9
ASC	37.5	43.8	46.7	65.6	68.8
AHBr	ND	ND	ND	ND	ND
HW	20.0	13.8	16.7	37.5	15.6

[a] ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; AHBr, activated hydrobromic acid; HW, hot water; ND, not determined.

Conclusions

A study was conducted to determine whether antimicrobial compounds currently used by the meat industry are effective against non-O157 STEC serogroups O26, O103, O111 and O145 compared to *E. coli* O157:H7. Based on these findings, all antimicrobial compounds used by meat industry appear to be effective against non-O157 STEC and the reductions of these pathogens on inoculated fresh beef were equally effective against *E. coli* O157:H7. The degree of effectiveness depended on the antimicrobial compounds used. In the present study, hot water was the most effective in reducing pathogens tested followed by lactic acid. Acidified sodium chlorite and activated hydrobromic acid showed less immediate effect, but increased effectiveness after chilling for 48 h at 4°C. Despite the reduced effect of these antimicrobial compounds, the recovery of these pathogens with low inoculation levels indicated that one of the following possibilities could occur: (a) the solutions might not be applied uniformly all of the surfaces since carcasses have irregular shapes and surfaces causing over-exposed to the treatment on one part and under-exposed on the other; (b) even with a uniform spray, all antimicrobial compounds will not only inactivate the bacterial cells, but also inflict sublethal injury to the cells. At a suitable environment, sublethal injured cells repair their injury, gain their normal characteristics, and subsequently initiate multiplication.

Presentation and Publication

A manuscript will be prepared for submission to a refereed scientific journal. Partial results of this study were presented at Reciprocal Meat Conference, June 21st, 2011 in Manhattan, KS.

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