Efficacy of commonly used antimicrobial interventions on Shiga toxin-producing *Escherichia coli* serotypes O45, O121, and non-MDR and MDR *Salmonella* inoculated fresh beef

Final report

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

Conclusions: 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 STECs and *Salmonella,* especially MDR strains versus non-MDR strains, is limited. A study was conducted to determine whether antimicrobial compounds currently used by the meat industry are effective against non-O157 STEC serogroups O45 and O121 compared to *E. coli* O157:H7 and against non-MDR and MDR *Salmonella*. All antimicrobial compounds that we tested were effective against non-O157 STEC and *Salmonella*. The reductions of these pathogens on inoculated fresh beef with tested compounds were at least as great as against *E. coli* O157:H7. The degree of effectiveness depended on the antimicrobial compounds used. In the present study, lactic acid, hot water, and acidified sodium chlorite were most effective in reducing the target pathogens. FreshFx had an intermediate effect in reducing pathogens, while peroxyacetic acid and BoviBrom had the least effect. The effectiveness of these compounds on non-MDR and MDR *Salmonella* was inconclusive, but Typhimurium were more resistant than Newport.

Deliverable: The results will assist the meat industry in identifying antimicrobial compounds suitable for controlling these pathogens in addition to *E. coli* O157:H7 and, thus, enhancing meat safety.

Technical Abstract

Ninety-six pre-rigor beef flank tissues (cutaneous trunci) were used to conduct a study to determine if antimicrobial interventions currently used by the meat industry are as effective in reducing non-O157 STEC serogroups O45, O121, and non-MDR and MDR *Salmonella* as they are at reducing *E. coli* O157:H7. Two inoculation levels, high (10^4 CFU/cm^2) and low (10^1 CFU/cm^2), of a ten-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%), BoviBrom (300 ppm), and hot water (85^{o}C). For samples inoculated with high bacterial concentrations, the remaining bacteria populations following each treatment were enumerated. For low level 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 lactic acid, hot water, and acidified sodium chlorite were effective in reducing STEC serogroups O45, O121, *E. coli* O157:H7, and *Salmonella* resulting in pathogen reductions of ≥ 2.3, ≥1.7, ≥1.5, and ≥ 1.5 log CFU/cm^2, respectively. Similar effectiveness also was found with low levels of inoculation on surface of beef flanks. FreshFx had an intermediate effect in reducing pathogens studied, while BoviBrom and peroxyacetic acid were the least effective in reducing pathogens. The results indicated that *Salmonella* Typhimurium were more resistant to the antimicrobial compounds than Newport. However, the effectiveness of these antimicrobial compounds on non-MDR and MDR of *Salmonella* was inconclusive. The results indicated that antimicrobial interventions used to reduce *E. coli* O157:H7 on fresh beef surfaces were equally effective against non-O157 STEC O45 and O121.

Introduction

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 may result in death. *Escherichia coli* O157:H7 and *Salmonella* are common human infectious agents throughout the world (Anonymous, 1997 and Glynn et al., 1998), and an estimated 1,400,000 (Mead et al., 1999) and 3,704 (Scallan et al., 2011) cases of *Salmonella* and *E. coli* O157:H7 infection, respectively occur in the U.S. annually. Both *Salmonella* and Shiga toxin-producing *E. coli* (STEC) have been found to contaminate carcasses at commercial beef processing facilities (Barkocy-Gallagher et al., 2003, Bosilevac et al., 2009, Brichta-Harhay et al., 2008). The prevalence of *Salmonella* on pre-evisceration carcasses was approximately 50 to 60% (Brichta-Harhay et al., 2008 and Bosilevac et al., 2009). In addition to *E. coli* O157:H7, there are other serotypes of STEC called "non-O157 STEC" that cause human diseases. CDC estimates that non-O157 STEC are responsible for about 1,579 confirmed cases of illness annually (Scallan et al., 2011). Non-O157 STEC can cause disease similar to that produced by *E. coli* O157:H7. More than 200 virulent non-O157 serotypes have been isolated from outbreaks, sporadic cases of HUS, and severe diarrhea in the U.S. and other countries (Brooks et al., 2005).

In the U.S., six O groups (comprising 13 serotypes) have been described by the CDC to be the cause of 71% of non-O157 STEC disease (Brooks et al., 2005). 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 may 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. The previous studies showed that the beef cattle hides and feces carried non-O157 STEC between 4.6 and 55.9%, representing a potential source of beef carcass contamination (Hussein, 2007). 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 about the same as the prevalence of *E. coli* O157:H7 (60.6%). The prevalence of non-O157 STECs (8%) was reported on carcasses after the application of multiple hurdle interventions (Arthur et al., 2002). Bosilevac et al. (2007) recently reported that the imported and domestic boneless beef trim used for ground beef in the U.S. had non-O157 STECs as high as 10 to 30%.

The USDA's National Animal Health Monitoring System (NAHMS) Feedlot '99 study showed that *Salmonella* fecal prevalence varied from 2.8 to 11.2%, with the peak prevalence occurring in the summer months (USDA, Animal and Plant Health Inspection Service [USDA-APHIS], 2001). U.S. dairy cattle have been shown to have a *Salmonella* fecal prevalence (7.4% of 3,669 samples) similar to fed cattle (USDA- APHIS, 2003). *Salmonella* have been found on the hides of fed beef cattle at higher rates than in feces. Hide prevalence varied from 27.7% in the winter to 91.6% and 97.7% in the summer and fall, respectively (Barkocy-Gallagher et al. 2003). Harhay et al. (2008) also found that the average prevalences of *Salmonella* on hides, preevisceration and postintervention carcasses were 89.6%, 50.2%, and 0.8%, respectively. Bosilevac et al. (2009) analyzed 4,136 ground beef samples collected from seven regions of the United States and reported that the overall prevalence rate of *Salmonella* strains was 4.2%. *Salmonella enterica* serovars Typhimurium and Newport are commonly identified in bovine clinical samples and in 2003 represented 11% and 30% of the reported bovine isolates respectively (CDC, 2004a). Salmonellosis in children is often invasive and requires antibiotic treatment for recovery. As such, it is concerning that in recent years, there has been a marked increase in the number of multi-drug resistant (MDR) *Salmonella* isolated in clinical settings. *S.* Typhimurium with the ACSSuT resistance pattern (ampicillin, chloramphenicol, streptomycin sulfisoxazole, and tetracycline), and *S.* Newport with the MDR-AmpC resistance pattern (Multi Drug Resistant *S.* Newport that are resistant to at least 9 of 17 antibiotics tested including: amoxicillin/clavulonic acid, ampicillin, cephalothin, ceftiofur, cefoxitin, ceftriaxone, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline) were two of the most common MDR *Salmonella* phenotypes isolated in 2002 (CDC, 2004b). *S.* Typhimurium DT-104 caused an outbreak in Northestern United States from August 2003 to January 2004 that was linked to commercial ground beef (Dechet et al., 2006).

Clearly, non-O157 STECs and *Salmonella* threaten consumers' health as well as cause economic loss due to illnesses, product condemnation, and lower product demand. In September 2011, FSIS published a Federal Register notice of their intent to regulate non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 as adulterants in certain raw beef products the same as *E. coli* O157:H7 beginning in March 2012. 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 STECs and *Salmonella* especially MDR strains versus non-MDR strains is limited.

Materials and Methods

Bacterial strains, growth conditions, and preparation of inoculum: Two strains each of non-O157 *E. coli* serotypes O45 :H2 (01E-1269 human isolate), O45 WDG3(isolated from cattle hide), O121:H19 (O1E-2074 human isolate) ,O121:H7 (isolated from ground beef), *E. coli* O157:H7 (ATCC 43895 and FSIS #4), *S.* Newport 15124 (non-MDR from cattle carcass), *S.* Newport 13109 (MDR from cattle carcass), *S.* Typhimurium 14218 (from cattle carcass), and *S.* Typhimurium (MDR, DT-104) from USMARC culture collection were grown for 16 to 18 h at 37°C in nutrient broth (Beckton Dickinson, Sparks, MD). Each strain was adjusted 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 was mixed to form a 10-strain cocktail mixture and diluted with purge. Purge was aseptically collected from vacuum packaged beef subprimals that had been stored at -20°C and then thawed at 4°C. The final concentrations of the purge containing cocktail mixtures were approximately 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 the 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 a 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 the cocktail mixture was inoculated on individual 25-cm^2 sections, spread over the area, and let stand 15 min at room temperature to allow bacterial cells attachment before subjecting the flanks 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 = 2.46; Ecolab, MN), (2) peroxyacetic acid (200 ppm, pH = 2.8; Ecolab), (3) FreshFx (1:50, pH = 1.77; SteriFX Inc., Shreveport, LA), (4) lactic acid (4%, pH = 2.2; PURAC, IL), (5) BoviBrom (300 ppm as HOBr, pH = 7.04; Elanco Food Solutions, Greenfield, IN), and (6) Hot water (85°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°C at nozzles) was sprayed at 15 psi, while the other antimicrobial compounds were freshly prepared with water (22 to 25°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°C before enumeration to determine residual effect on antimicrobial treatments. The other set of bags was enumerated within 10 min following the treatments.

Microbiological 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 differential U.S. Meat Animal Research Center (USMARC) chromogenic medium and were plated on non-selective medium for aerobic plate count (APC) using petrifilm (3M, St. Paul, MN). 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. 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 O45, O121, and *E. coli* O157:H7 were picked for confirmation using multiplex PCR (Perelle et al., 2004). For *Salmonella*, colonies were picked for confirmation as Newport or Typhimurium using multiplex PCR (Kim et al., 2006). 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 (IMS) of target organisms. One milliliter aliquot of each enriched sample was subjected to IMS as described previously (Barkocy-Gallagher et al., 2003 and Nou et al., 2006). The bacterial bead complexes were spread plated on CHROMAgar O157™ (DRG International, Mountainside, NJ, supplemented with 5 mg of novobiocin/L and 1.0 mg of potassium tellurite/L; ntCHROMAgar) for *E. coli* O157:H7. For *Salmonella*, the bacterial bead complexes were subjected to secondary enrichment with 3-ml of Rappaport-Vassiliadis soya peptone broth and incubated at 42°C for 24 h before streaking for isolation on Xylose Lysine Deoxycholate agar (XLD; Becton Dickinson). Both ntCHROMAgar and XLD plates were incubated at 37°C for 24 h. Four colonies from each sample were picked into 1-ml of tryptic soy broth (Becton Dickinson) and incubated for 24 h at 37°C for PCR (Kim et al 2006) to determine for Newport or Typhimurium and for culturing on tryptic soy agar supplemented with 32 mg of tetracycline/liter (TSAtet) to determine non-MDR or MDR strains. Since there is no commercial immunomagnetic bead for serogroup O45 and O121, an aliquot of 20-µl 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 that have color characteristics for each serogroup were picked for confirmation using multiplex PCR (Perelle et al., 2004).

Statistical analyses: 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 squares means were 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 previous studies, due to lack of methodological detail. 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, lactic acid, BoviBrom, and hot water is presented in Table 1. In general, all six antimicrobial compounds were effective in reducing the population of O45, O121, *E. coli* O157:H7, and *Salmonella* on fresh beef. Acidified sodium chlorite reduced ($P < 0.05$) serogroups O45, O121, *E. coli* O157:H7, and *Salmonella* from 4.5 to 2.6, 3.9 to 1.9, 4.5 to 2.6, and 4.7 to 3.2 log CFU/cm^2, respectively, following spray treatment. Similar reductions were observed in *Salmonella* and *E. coli* O157:H7 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 O45, O121, *E. coli* O157:H7, and *Salmonella* from 2.6 to 2.1, 1.9 to 1.1, 2.6 to 2.2, and 3.2 to 2.6 log CFU/cm^2, respectively.

Spray treatment with peroxyacetic acid at 200 ppm immediately reduced ($P < 0.05$) the population of O45, O121, *E. coli* O157:H7, and *Salmonella* on surface of beef flanks from 4.4 to 3.4, 4.1 to 3.0, 4.4 to 3.5, and 4.5 to 3.6 log CFU/cm^2, respectively. Similar results of spray treatment with peroxyacetic acid on *E. coli* O157:H7 inoculated beef carcasses have 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 had 0.7 additional log reduction on O121, *E. coli* O157, and *Salmonella*, but O45 was not further reduced after chilling.

FreshFx reduced ($P < 0.05$) the population of STEC O45, O121, and *Salmonella* on inoculated fresh beef from 4.4 to 3.3, 4.2 to 2.7, and 5.0 to 3.3 log CFU/cm^2, respectively. FreshFx was reported to have a similar antimicrobial effect on reduction of *E. coli* O157:H7 (Kalchayanand et al., 2008). FreshFx showed no additional reduction on O45, O121, and *Salmonella* after chilling.

Treatment with 4% lactic acid reduced ($P < 0.05$) non-O157 STEC serogroups O45, O121, *E. coli* O157:H7, and *Salmonella* from 4.2 to 1.7, 4.3 to 1.6, 4.2 to 1.9, and 4.6 to 1.5 log CFU/cm^2, respectively. The effect of lactic acid on the reduction of *E. coli* O157:H7 inoculated cheek meat was reported when spray treated with lactic acid (Kalchayanand et al., 2008). 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). The approximately 0.5 additional log reduction ($P < 0.05$) for *E. coli* serogroups tested and on *Salmonella* after chilling 48 h at 4^{o}C following lactic acid treatment was not significant.

BoviBrom is a commercial trade name for a compound called 1, 3- Dibromo-5, 5 dimethylhydantoin (DBDMH). In aqueous solution, DBDMH hydrolyses to hydrobromoue acid (HOBr), which is a biocide (Sun et al., 1995). BoviBrom (pH = 7.0) reduced ($P < 0.05$) STEC O45, O121, *E. coli* O157:H7, and *Salmonella* on inoculated beef flanks from 4.4 to 3.3, 3.9 to 2.5, 4.5 to 3.5, and 4.9 to 4.0 log CFU/cm^2, respectively. In a previous study, DBDMH reduced O157:H7 inoculated fresh beef and beef heart 1.6 to 2.1 log reductions (Kalchayanand et al., 2009). This discrepancy was determined to depend on the amount of selective agent used in the plating medium. The study in 2009 used medium with high concentration of selective agent to reduce the background flora due to the study involved with fecal materials. The stressed target organisms from the treatment could not multiply on the medium with high selective agent. We compared HOBr (300 ppm) generated from hydrobromic acid or DBDMH and did not find significantly difference in the reduction of *E. coli* O157:H7 and other STEC strains. The reduction of the tested strains depended on the concentration of selective agent incorporated in the medium (data not shown). When BoviBrom treated samples were chilled for 48 h at 4^{o}C, the population of *E. coli* O157:H7 and *Salmonella* were reduced ($P < 0.05$) from 3.5to 3.1 and 4.0 to 3.7 log CFU/cm^2, respectively. There was no additional reduction for O45 and O121.

Hot water treatment has been found to be 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). In the present study, the reductions of all STEC O45, O121, and *Salmonella* also were observed when hot water was used as antimicrobial intervention. Hot water reduced ($P < 0.05$) aforementioned STEC and *Salmonella* from 4.0 to 1.6, 3.9 to 2.2, and 4.9 to 3.0 log CFU/cm^2, respectively. Although the present study did not include *E. coli* O157:H7, the reductions for tested strains were lower than expected. It was determined that in the present study, the hot water safety valve did not work properly and caused water temperature drop during spray treatment. 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.

Although acidified sodium chlorite, peroxyacetic acid, FreshFx, lactic acid, BoviBrom, and hot water were generally able to reduce population of STEC (Table 1), 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 2. Overall, antimicrobial compounds used in meat industry reduce STEC strains O45, O121, *E. coli* O157:H7, and *Salmonella* following the treatments ranging from 0.8 to 3.1 log CFU/cm^2 on fresh beef. Acidified sodium chloride reduced STEC ranging from 1.5 to 2.0 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 approximately 1.0 log reduction. FreshFx significantly reduced ($P < 0.05$) serotype O45, O121, and *Salmonella* ranging from 1.1 to 1.7 log reduction. 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 2.3 to 2.7 log reductions, where *Salmonella* was the most sensitive to lactic acid (3.1 log reductions).

Both BoviBrom and hot water were neutral antimicrobial compounds used in this study and significantly reduced ($P \leq 0.05$) STEC strains O45, O121, *E. coli* O157:H7, and *Salmonella*. BoviBrom reduced STEC ranging from 0.8 to 1.3 log reduction, which *Salmonella* was the least sensitive to the treatment compared to non-O157 STEC serogroups (Table 2). Less sensitivity of *Salmonella* to HOBr compared to *E. coli* O157:H7 also was reported on beef flanks and beef hearts treated with HOBr (Kalchayanand et al., 2008). Hot water reduced STEC on inoculated beef flanks ranging from 1.9 to 2.4 log reduction. Based on the enumeration on selective chromogenic medium, lactic acid and hot water were the most effective in reducing serogroups O45, O121, *E. coli* O157:H7, and *Salmonella* followed by acidified sodium chlorite and FreshFx. Peroxyacetic acid and BoviBrom seemed to have equal effect on tested pathogens on inoculated fresh beef.

Antimicrobial treatments not only inactivate but also inflict sublethal injury to microorganisms. Therefore, using selective medium to enumerate may lead to overestimating 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 a non-selective medium. Non-selective media (aerobic count plate, 3M) allows sublethally injured cells to resuscitate and grow. The efficacy of antimicrobial compounds tested on aerobic plate counts (APC) is presented in Figure 1. The inactivation ranged from 0.8 to 1.9 log reduction, with lactic acid, hot water, and acidified sodium chlorite being more effective in reducing total bacteria counts than FreshFx, peroxyacetic acid, and BoviBrom. 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 in treated ground beef.

The advantage of using USMARC chromogenic medium is that the medium supports growth of *Salmonella* and the colony color can be distinguished among the STEC strains. *Salmonella* colonies were picked for multiplex PCR and culturing on TSAtet to determine their serotypes and which serotypes were non-MDR and MDR strains. Lactic acid, hot water, and acidified sodium chlorite were more effective in reducing *Salmonella* than FreshFx, peroxyacetic acid, and BoviBrom (Table 3). The results were reported as percent survival compared to untreated control. A higher percentage of survivors, reflects greater resistance to the interventions. In general, Typhimurium showed more resistance to the antimicrobial treatments than Newport. Newport survived treatments ranging from 0 to 74.2%, while Typhimurium survival ranged from 2.2 to 77.5%. However, when *Salmonella* strains were broken down into non-MDR and MDR, the MDR Newport strain showed more resistance to antimicrobial compounds (except for lactic acid) than non-MDR Newport strain. In contrast, non-MDR Typhimurium showed more resistance to treatment compounds (except acidified sodium chlorite and hot water) than MDR Typhimurium strains. More studies need to be conducted to pinpoint the effectiveness these antimicrobial compounds are for inactivation of non-MDR and MDR *Salmonella*.

Low inoculation study: The efficacy of acidified sodium chlorite, peroxyacetic acid, FreshFx, lactic acid, BoviBrom, 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°C were calculated and are presented in Table 4. The recovery rate of STEC serogroup O45, O121, *E. coli* O157:H7, and *Salmonella* ranged from18.2 to 100.0%. Lactic acid, hot water and acidified sodium chlorite were more effective in reducing *E. coli* O157:H7, non-O157 STEC O45 and O121, and *Salmonella*. Since there are no anti-nonO157 STEC O45 and O121 immunomagnetic beads commercially available, it was very difficult to identify these two serogroups from the enriched samples and the results of recovery of O45 and O121 may be underestimated.

Conclusions

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 STECs and *Salmonella* especially MDR strains is limited. A study was conducted to determine whether antimicrobial compounds currently used by the meat industry are effective against non-O157 STEC serogroups O45 and O121 compared to *E. coli* O157:H7 and their effectiveness on non-MDR and MDR *Salmonella*. Based on these findings, all antimicrobial compounds used by the beef industry were effective against non-O157 STEC and *Salmonella*. The reductions of these pathogens on inoculated fresh beef with tested compounds in general, were as effective against non-O157 STECs as *E. coli* O157:H7. The degree of effectiveness depended on the antimicrobial compounds used. In the present study, lactic acid, hot water, and acidified sodium chlorite were more effective in reducing pathogens. FreshFx had an intermediate effect, while peroxyacedtic acid and BoviBrom had the least effect. The effectiveness of these compounds on non-MDR and MDR was inconclusive and possibly strain dependent, but Typhimurium were more resistant than Newport. 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 to all of the surfaces as carcasses have irregular shapes and surfaces causing over-exposure to the treatment on one part and under-exposure on others; (b) even with a uniform spray, all antimicrobial compounds will not only inactivate the bacterial cells, but also inflict sublethal injury to the cells. An enumeration with selective medium may overestimate effects of the antimicrobial interventions used. It should be noted that in a suitable environment, sublethally injured cells may 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.
Presentation: "Prevalence of non-O157 STEC and the Efficacy of Interventions" at the Reciprocal Meat Conference, Manhattan, KS, June, 2011.

Table 1. Effectiveness of antimicrobial compounds in reducing non-O157 STEC and *E. coli* O157:H7 at high inoculation levels.

Treatment[a]	Survivors of STEC on selective medium[b] (log CFU/cm^2)			
	O45	O121	O157	Sal
Control	4.5^A	3.9^A	4.5^A	4.7^A
ASC	2.6^B	1.9^B	2.6^B	3.2^B
$ASC_{chilled}$	2.1^B	1.1^C	2.2^B	2.6^C
Control	4.4^A	4.1^A	4.4^A	4.5^A
POA	3.4^B	3.0^B	3.5^B	3.6^B
$POA_{chilled}$	3.3^B	2.3^C	2.8^C	2.9^C
Control	4.4^A	4.2^A	ND	5.0^A
Fx	3.3^B	2.7^B	ND	3.3^B
$Fx_{chilled}$	3.2^B	2.7^B	ND	3.2^B
Control	4.2^A	4.3^A	4.2^A	4.6^A
LA	1.7^B	1.6^B	1.9^B	1.5^B
$LA_{chilled}$	1.1^B	1.0^B	1.3^B	1.1^B
Control	4.4^A	3.9^A	4.5^A	4.9^A
BB	3.3^B	2.5^B	3.5^B	4.0^B
$BB_{chilled}$	3.2^B	2.1^B	3.1^C	3.7^C
Control	4.0^A	3.9^A	ND	4.9^A
HW*	1.6^B	2.2^B	ND	3.0^B
$HW_{chilled}$	1.5^B	1.8^B	ND	3.1^B

[a]Control, inoculated and sampled without any treatment; ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; BB, BoviBrom; 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. Sal = *Salmonella.*

*The safety valve did not work properly causing temperature drop during hot water treatment.

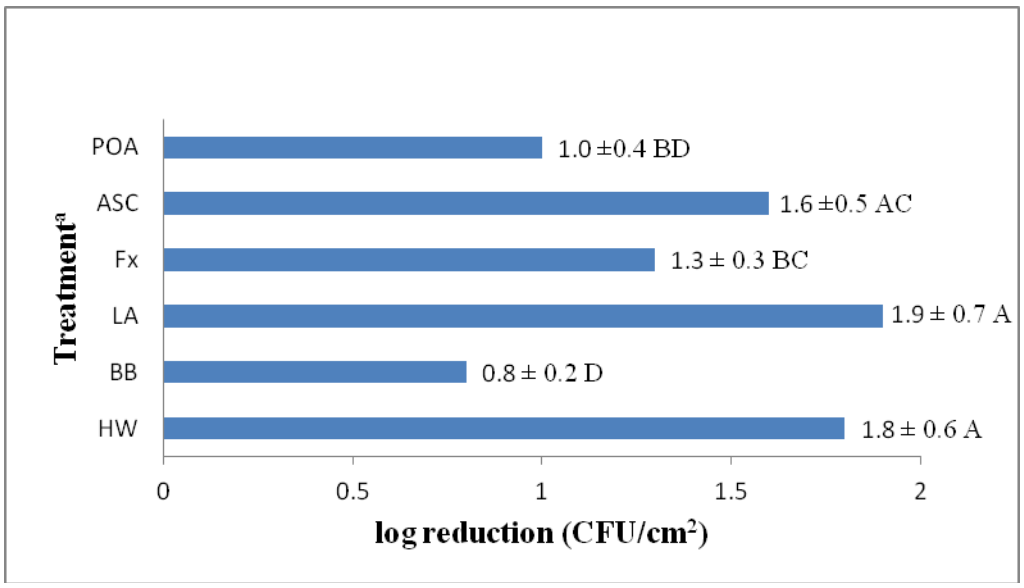
Within a treatment type, means with no common letter that are in the same column are significantly different ($P \leq 0.05$).

Table 2. Compare inactivation efficiency of antimicrobial compounds between non-O157 STEC and *E. coli* O157:H7 at high inoculation levels.

Treatment[a]	Reduction of STEC on selective medium[b] (log CFU/cm^2)			
	O45	O121	O157	Sal
ASC	1.9^{A}	2.0^{A}	1.8^{A}	1.5^{A}
POA	0.9^{A}	1.1^{A}	0.9^{A}	0.9^{A}
Fx	1.1^{A}	1.5^{AC}	ND	1.7^{BC}
LA	2.6^{AB}	2.7^{AB}	2.3^{B}	3.1^{A}
BB	1.1^{A}	1.3^{A}	1.0^{A}	0.8^{B}
HW*	2.4^{A}	1.7^{B}	ND	1.9^{AB}

[a]ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; BB, BoviBrom; HW, hot water. Each treatment, n = 32.
[b]USMARC chromogenic medium. Sal = *Salmonella.*
*The safety valve did not work properly causing temperature drop during hot water treatment.
Within a treatment type, means with no common letter that are in the same row are significantly different ($P \leq 0.05$).

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



POA, peroxyacetic acid; ASC, acidified sodium chlorite; Fx, FreshFx; LA, lactic acid; BB, BoviBrom; HW, hot water. Each treatment, n =32.
A-D, means bearing with no common letter are significantly different ($P \leq 0.05$)

Table 3. *Salmonella* survivors on USMARC chromogenic medium after intervention treatments for high inoculation levels.

	% Survival relative to untreated control			
	Newport		Typhimurium	
Treatment[a]	Non-MDR	MDR	Non-MDR	MDR
POA	3.7	14.8	77.5	43.8
ASC	0	53.6	47.8	44.9
FX	10.2	29.6	66.3	52.3
LA	0	0	12.2	2.2
BB	23.7	65.8	64.8	38.6
HW	3.2	74.2	29.2	24.6

[a]ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; BB, BoviBrom; HW, hot water. Each treatment, n = 126.

Table 4. Recovery of STEC and *Salmonella* after intervention treatments and chilled for 48 h at 4°C for low inoculation levels.

	% recovery relative to untreated controls			
Treatment[a]	O45	O121	O157	Sal
POA	53.6	55.6	75.0	81.2
ASC	53.8	38.5	61.3	73.3
FX	62.5	14.3	ND	78.1
LA	18.2	21.0	19.0	20.7
BB	89.3	19.2	76.7	100.0
HW	37.5	53.1	ND	81.2

[a]ASC, acidified sodium chlorite; POA, peroxyacetic acid; Fx, FreshFx; LA, lactic acid; BB, BoviBrom; HW, hot water. Each treatment n =32.
Sal = *Salmonella*

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