Antimicrobial Interventions for O157:H7 and Non-O157 Shiga Toxin–Producing *Escherichia coli* on Beef Subprimal and Mechanically Tenderized Steaks

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**ABSTRACT**

Non-O157 Shiga toxin–producing *Escherichia coli* (STEC) is an emerging risk for food safety. Although numerous postharvest antimicrobial interventions have been effectively used to control *E. coli* O157:H7 during beef harvesting, research regarding their effectiveness against non-O157 STEC is scarce. The objectives of this study were (i) to evaluate effects of the spray treatments—ambient water, 5% lactic acid (LA), 200 ppm of hypobromous acid (HA), and 200 ppm of peroxyacetic acid (PA)—on the reduction of O157:H7 or non-O157 STEC (O26, O103, O111, and O145) with high (10⁶ log CFU/50 cm²) or low (10⁴ log CFU/50 cm²) levels on beef subprimals after vacuum storage for 14 days and (ii) to evaluate the association of the antimicrobial treatments and cooking (50 or 70 °C) on the reduction of the pathogens in blade-tenderized steaks. The treatment effects were only observed (*P* = 0.012) on samples taken immediately after spray intervention treatment following inoculation with a high level of O157:H7. The LA and PA treatments significantly reduced low-inoculated non-O157 STEC after spray intervention; further, the LA and HA treatments resulted in significant reductions of non-O157 STEC on the low-inoculated samples after storage. Although cooking effectively reduced the detection of pathogens in internal steak samples, internalized *E. coli* O157:H7 and non-O157 STEC were able to survive in steaks cooked to a medium degree of doneness (70 °C). This study indicated that the reduction on surface populations was not sufficient enough to eliminate the pathogen’s detection following vacuum storage, mechanical tenderization, and cooking. Nevertheless, the findings of this study emphasize the necessity for a multihurdle approach and further investigations of factors that may influence thermal tolerance of internalized pathogenic STEC.

Shiga toxin–producing *Escherichia coli* (STEC) has been recognized as a human pathogen since 1982 following the outbreaks associated with *E. coli* O157:H7 in contaminated hamburger patties in the United States (25). Since then, *E. coli* O157:H7 has been the most prevalent and virulent STEC associated with various foodborne outbreaks in the United States (26). However, in some instances, non-O157 STEC strains have been implicated in human illness and seem to be capable of causing disease as severe as that caused by *E. coli* O157:H7 (4, 21). The recent rise in non-O157 STEC illnesses is likely due to the development and improved sensitivity of new screening technologies (30). Due to the potential cause of severe human illness by these organisms, the U.S. Department of Agriculture, Food Safety and Inspection Service has declared six of the STEC serogroups (O26, O45, O103, O111, O121, and O145) as adulterants in certain beef products, such as raw ground beef and tenderized beef (32).

Because cattle are a major reservoir of *E. coli* O157:H7 and non-O157 STEC strains, it is very hard to eradicate these microorganisms (12, 24). It has been demonstrated that these microorganisms can be transmitted to a carcass surface from bovine hides and feces during beef processing (29). Therefore, it is not only important to maintain equipment sanitation, such as knives and cutting boards, but also to reduce bacterial loads on carcasses to assure the safety of final products. In regard to fresh meat, cross-contamination may occur at multiple points during further processing. Mechanical tenderization, for example, is a common practice used to improve meat tenderness via mechanical manipulation of the muscle tissues with needles or blades (10, 17). Though successful at improving palatability, the process introduces the risk of translocating surface bacteria into deep tissues that were previously considered sterile (10). Additionally, previous research indicated that cooking to 71 °C may not eliminate internalized *E. coli* O157:H7 and non-O157 STEC in the tenderized steaks (19). This risk is exacerbated because tenderized, or nonintact, steaks are generally not distinguishable in appearance and are frequently prepared in a similar way to intact steaks by food handlers and consumers. The failure of inactivating the internalized pathogens by further processing, such as cooking, could pose a risk for human
health. Thus, it is critical to control surface contamination from carcasses to wholesale subprimal cuts and, thereby, minimize the translocation of pathogenic microorganisms into the center of meat to assure the safety of tenderized beef.

To prevent cross-contamination by decreasing surface bacterial concentrations, antimicrobials have been widely used on a beef carcass during beef harvesting. Previous studies reported that the prevalence of *E. coli* O157 and non-O157 STEC together on a beef carcass was reduced from approximately 70 to 10% after application of antimicrobial interventions (3, 7). Furthermore, the efficacies of using weak acids as antimicrobial treatments, such as acetic acid and lactic acid (LA) solution, on beef carcasses to decrease contamination of *E. coli* O157:H7 or *Salmonella* during beef processing have also been documented (9, 16). Recently, several studies reported that intervention strategies currently used to prevent *E. coli* O157:H7 on a beef surface were likely to be as effective against non-O157 STEC (15, 21). However, the research concerning the use of antimicrobial interventions to reduce a cocktail of non-O157 STEC strains is relatively scarce. In addition, the effects of multiple interventions to control STEC is not well known. Thus, the objectives of this study were (i) to evaluate effects of the weak acid antimicrobial spray treatments on the reduction of *E. coli* O157:H7 or non-O157 STEC (serogroups O26, O103, O111, and O145) on beef subprimal carcasses after vacuum storage at 4°C for 14 days and (ii) to evaluate the association of the antimicrobial treatments and cooking (50 or 70°C) on the reduction of the pathogens in blade-tenderized steaks.

**MATERIALS AND METHODS**

**Meat samples.** Beef strip loin subprimals (USDA Select, Institutional Meat Purchase Specifications no. 180 (31)) were obtained approximately 7 days postmortem from a federally inspected commercial processing facility and transported to Texas Tech University (Lubbock). All subprimals were tested at the processing facility to ensure that there was no background presence of pathogens, such as *E. coli* O157:H7 and STEC. Subprimals remained at the pathogen processing facility for inoculation and further processing.

**Strains.** Inoculum cocktail for the *E. coli* O157:H7 portion of the trial was obtained using four strains (ATCC no.: A4 966, A5 528, 966, and A1 920) previously associated with Centers for Disease Control and Prevention–documented outbreaks of animal origin. Non-O157 STEC cocktails were obtained from each of non-O157 STEC (*E. coli* O26:H11: ATCC BAA-1653; O103:H8; ATCC 23982; O111:H8; ATCC BAA-181; and O145:H28: ATCC BAA-1652). In addition to *E. coli* O157:H7, four non-O157 STEC strains (O26, O103, O111, and O145) were selected because immunomagnetic beads (Dynal, Lake Success, NY) for serogroups O45 and O121 were not yet commercially available at the time of experimentation. These non-O157 strains were from either human stools that had also been associated with human illness or cattle feces.

**Cocktail preparation.** Each frozen strain of *E. coli* O157:H7 or non-O157 STEC was thawed and individually inoculated into 9 ml of tryptic soy broth (TSB; Difco, BD Microbiology Systems, Sparks, MD) and incubated at 37°C for 24 h. On the following day, the enriched cultures were transferred to 9 ml of TSB (Difco, BD tubes and incubated at 37°C for 24 h, and the concentration of the enriched cultures was determined by direct plating method. The same enriched process was conducted, as well as culture concentration on the third day. Subsequently, an equal amount of enrichment from each strain was compiled to make cocktails that were dispensed in 1-ml frozen vials with 10% (vol/vol) sterile glycol as a cryoprotectant and stored at ~ 80°C. The inoculum was prepared by growing a frozen vial of cocktail in 99 ml of TSB at 37°C for 24 h to make 3 liters for each concentration at 1 × 10⁸ CFU/liter (high level) and 1 × 10⁴ CFU/liter (low level).

**Inoculation.** *E. coli* O157:H7 and non-O157 STEC inoculation occurred separately. Prior to inoculation, the beef strip loins (n = 15; average weight = 4.5 kg) were split into two equal portions that were evenly and randomly assigned to either inoculation with the high- or low-level cocktail (n = 15 per inoculation serogroup, per replication). The subprimal sections were submersed in a polypropylene container filled with 3 liters of inoculum for 1 min (30 s per dorsal and ventral side) to obtain a surface concentration of 1 × 10⁷ CFU/50 cm² or 1 × 10⁵ CFU/50 cm². After submersion, subprimals were placed lean side up on metal racks and held at 4°C for 30 min to facilitate bacterial attachment on meat surfaces.

**Spray intervention treatments and storage at 4°C.** Following bacterial attachment, inoculated beef subprimals were subjected to one of the following antimicrobial treatments: no treatment (CTRL), sterilized ambient water (AW; 21 ±1°C), 5% LA (Birko Corp., Henderson, CO), 200 ppm of hypobromous acid (HA; Birko Corp.), and 200 ppm of peroxycetic acid (PA; Birko Corp.). The LA, HA, and PA solutions were prepared using stock acids and potable water at room temperature by a Birko technician, according to the manufacturer’s instructions, shortly before use. Spray interventions were applied in a room maintained at ambient temperature (23°C) by using a customized stainless steel spray cabinet (Chad Co., Olathe, KS) with a conveyorized wire belt. The spray cabinet housed six nozzles, with a spray rate of 0.66 gal (2.5 liters) per min at 20 lb/m² and processed, as described previously (13). The spray cabinet and all production equipment were cleaned and sanitized between treatments.

After spray treatment, beef subprimal halves were placed on metal racks to drain for 5 min (4°C). Subprimals were then individually vacuum packaged in a high barrier bag (Cryovac, Sealed Air Corporation, Bolingbrook, IL) by using a vacuum machine (model MVS 4S, Minipack-Torre, Dublin, UK) and stored in the dark at 4°C for 14 days. The temperature of the refrigerator used to store the samples was monitored continuously throughout the storage period by using remote temperature probes (model SR1, Sapac, Modesto, CA).

**Mechanical tenderization.** After 14 days of vacuum storage, beef subprimal halves from each treatment and inoculation level were mechanically tenderized using a blade tenderization unit (model H, Jaccard, Orchard Park, NY). Individual subprimals per treatment and inoculation level were placed lean side up and penetrated with blades. The tenderization unit was appropriately disassembled, cleaned, and sanitized between each treatment group and inoculation level. After tenderization, the subprimals were portioned into three, 2.54-cm-thick steaks that were placed in sterile plastic bags and transported in an insulated cooler to the Experimental Sciences Laboratory at Texas Tech University (Lubbock) for further analysis.
Cooking procedure. The three steaks from each subprimal were randomly assigned to one of three internal cooking temperatures: uncooked, 50°C, or 70°C. The temperatures (50 or 70°C) were chosen based on degree of doneness for steaks. To accurately monitor the internal cooking temperature throughout the cooking period, for steaks assigned to 50 and 70°C, a thermocouple wire (Type J; Cole Parmer, Vernon Hills, IL) connected to a data monitoring device (Personal Daq, model OMB-DAQ-56, Omega Engineering, Inc., Stamford, CT) was placed in the geometric center of the steak, as recommended in research cooking guidelines provided by the American Meat Science Association (2). Steaks were cooked on clamp-style grills (model GRP99B, George Foreman, Salton Inc., Mount Prospect, IL), preheated to a surface temperature of 195°C. Steaks were removed from the grill using sterile tongs at their targeted internal temperature and allowed to rest at room temperature for 5 min. Due to a carryover-cooking factor, the temperature keeps increasing; therefore, a peak temperature was recorded.

Microbiological analysis: sample collection. In the first replication of E. coli O157:H7 for both high and low inoculation, a swab sample was collected from one of three individual beef subprimal half. For the remaining two replications of the E. coli O157:H7 study and all three replications of the non-O157 STEC study, swab samples were collected from each of three beef subprimal halves. The reason to include swab samples from all three beef subprimal halves instead of one was to facilitate data analysis. Swab samples were collected from the lean side of the subprimal half 5 min after spray intervention by using a sponge preimmersed with 10 ml of buffered peptone water (BPW) and a sterile 50-cm² area template and after 14 days of refrigerated storage prior to tenderization.

For steak samples, a 50-cm² surface swab was taken from one steak, assigned to uncooked portions, to determine internalized bacterial levels through a needle tenderization process. Additionally, a portion of meat (core sample) with an area (2 by 5 cm) was removed from the geometric center of each steak (uncooked or cooked to 50 and 70°C) by cutting through the whole thickness of the steak using a sterile template and knife. The core samples contained two sides of steak surfaces. The geometric center is the location in which the temperature was taken and the most likely location for pathogen survival in the steak itself after cooling. The sample was ground for 1 min using a food processor (HC306, Black and Decker, Townson, MD). Ten grams of the core sample was collected and placed into a stomacher bag (3M, St. Paul, MN) with 90 ml of Difco Gram-Negative (GN) broth (BD, Franklin Lakes, NJ) for E. coli O157:H7 or BPW for non-O157 STEC for further analysis.

E. coli O157:H7 samples. Swab samples were homogenized for 1 min in 10 ml of BPW, whereas core samples were homogenized for 2 min with 90 ml of Difco GN broth using a stomacher (Seward Stomacher 400, West Sussex, UK) at a speed of 230 rpm. Swab samples were serially diluted in BPW; following serial dilutions, 100 or 250 μl of the diluted sample was spread onto MacConkey’s agar (BD) supplemented with potassium tellurite (2.5 mg/liter) and novobioin sodium salt (2.5 mg/liter). Plates were inverted and incubated at 37°C for 18 (±2) h for enumeration. Colonies of E. coli O157:H7 were then visually counted. If pathogen levels were below detection level by direct plating, 1 ml of sample was taken from each swab and core sample bag and placed into a tube with 9 ml of Difco GN broth and then incubated for an extra 6 h at 42°C. Next, the enrichments were subjected to immunomagnetic separation assay. One milliliter of each enriched sample was mixed with 1 ml of phosphate-buffered saline Tween 20, pH 7.4 (Sigma-Aldrich, St. Louis, MO) and 20 μl of anti-O157 Dynabeads (Dynal) and then analyzed using an automatic immunomagnetic separation assay BeadRetriever instrument (Dynal). Subsequently, 50 μl of the bead-bacteria mixture of E. coli O157 was plated onto CHROMagar O157 (CHROMagar Microbiology, Paris, France) with potassium tellurite (2.5 mg/liter) and novobioin sodium salt (2.5 mg/liter) and incubated at 37°C overnight. Suspect colonies with a mauve color were selected and subjected to a latex agglutination test containing O157 antigens (Oxoid, Cambridge, UK) for confirmation.

Non-O157 STEC samples. Swab samples obtained from non-O157 inoculated subprimals were homogenized for 1 min in 10 ml of BPW at 230 rpm, whereas core samples were homogenized for 2 min in 90 ml of BPW (Oxoid Ltd., Basingstoke, Hampshire, UK). Afterwards, serial dilutions were formed, and 100 or 250 μl of the diluted sample was plated onto Possé agar (23) and incubated at 37°C for 18 to 24 h. Bacterial colonies were then visually counted. If pathogen levels were below detection level by direct plating, 1 ml of sample was taken from each swab and core sample bag and placed into a tube with 9 ml of BPW (3M) and then incubated for 6 h at 37°C. Next, from each sample, 50 μl were transferred into separate screw-top lysis tubes (Pall GeneDisc Technologies, Pall Life Sciences, Ann Arbor, MI) and placed in a boiling water bath (100°C) for 10 min to allow cell lysis. DNA was extracted from the lysis tubes, loaded onto the GeneDisc plates (Pall GeneDisc Technologies), according to the manufacturer’s instructions, and analyzed by using a closed-platform PCR system (Pall GeneDisc Technologies) to detect the target bacteria.

Statistical analyses. E. coli O157:H7 and non-O157 STEC were conducted separately. Each organism was replicated three times. Evaluations of E. coli O157:H7 and non-O157 STEC were arranged as separate incomplete block experiments that were replicated on three independent occasions. Additionally, high- and low-level concentrations of inocula were treated as independent trials and were evaluated separately. Pathogen populations on subprimal and steak surfaces as influenced by spray intervention treatment and days of storage (0 or 14 days subprimal only) were assessed using a linear mixed model. The geometric mean of the populations within each replication were calculated prior to statistical analysis. Spray intervention treatment, time (days 0 and 14), and their interaction were included as fixed effects, while replication was incorporated in the model as a random effect. Data were analyzed using a commercial statistical software package (SAS, version 9.3, Cary, NC), and model-adjusted estimates of concentration were computed for each level of the independent variable. Differences in the detection of the O-antigen as assessed by PCR-based methodologies and influenced by cooking temperature (50 or 70°C), and spray intervention treatment in cooked internal steak cores was determined using a generalized linear mixed model with cooking temperature, intervention treatment, and their interaction as fixed effects, while replication was treated as a random effect.

RESULTS AND DISCUSSION

Subprimal surface bacterial populations. For high-inoculated E. coli O157:H7 on the surface, spray treatment with AW, LA, HA, and PA resulted in a reduction (P = 0.012) of surface populations on day 0 when compared with the CTRL (Table 1). No differences (P > 0.05) were
observed among the acid-based treatments (HA, PA, and LA) or AW. On day 14, treatment effect had no influence (P = 0.209) on surface subprimal populations (Table 1). At low inoculation, spray intervention, regardless of treatment, did not significantly decrease (P = 0.179) E. coli O157:H7 on beef surfaces on day 0. After storage, no difference of bacterial population was observed on the meat surfaces. However, for both inoculations, the levels of surface bacteria among all groups were significantly reduced during storage by approximately 2 and 1 log CFU/50 cm² bacteria among all groups were significantly reduced during storage. Specifically, the HA- and PA-treated subprimals had significant fewer cells compared with other treatments, such as LA and PA, used for water treatment on the reduction of surface levels and microorganisms. Wolf et al. (33) indicated that water spray treatment minimally reduced E. coli O157:H7 and non-O157 STEC on chilled beef surface, whereas Cutter and Rivera-Betancourt (5) found a substantial reduction of these organisms by water treatment through spray interventions. Additionally, the antimicrobial effect of water could be influenced by the time, pressure, and temperature at which it was applied (11).

Overall, the results of this study showed that the effects of AW treatment on the reduction of surface E. coli O157:H7 on day 0 varied according to different inoculation levels and microorganisms. Wolf et al. (33) indicated that water spray treatment minimally reduced E. coli O157:H7 and non-O157 STEC on chilled beef surface, whereas Cutter and Rivera-Betancourt (5) found a substantial reduction of these organisms by water treatment through spray interventions. Additionally, the antimicrobial effect of water could be influenced by the time, pressure, and temperature at which it was applied (11).

Kalchayanand et al. (15) reported that antimicrobial treatments, such as LA and PA, used for E. coli O157:H7 were at least as effective for non-O157 STEC on prerigor beef; however, PA had a lower antimicrobial activity than LA. Furthermore, the effectiveness of PA varied in different studies. Penney et al. (22) reported substantial antimicrobial activity of PA against E. coli O157:H7 on the external carcass surfaces of prerigor beef inoculated with fecal contamination. However, other studies demonstrated less effectiveness of the PA treatment against coliforms or E. coli O157:H7 on either

**TABLE 1. Levels of E. coli O157:H7 recovered from beef subprimal surfaces immediately after antimicrobial spray treatment and after 14 days of storage at 4°C**

<table>
<thead>
<tr>
<th>Inoculum concn</th>
<th>Spray treatment</th>
<th>Log CFU/50 cm²</th>
<th>Day 0&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 14&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>High&lt;sup&gt;d&lt;/sup&gt;</td>
<td>CTRL</td>
<td>6.27 a A</td>
<td>4.25 a B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AW</td>
<td>6.02 b A</td>
<td>3.89 a B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% LA</td>
<td>5.79 b A</td>
<td>3.63 a B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of HA</td>
<td>5.88 b A</td>
<td>3.88 a B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of PA</td>
<td>5.90 b A</td>
<td>4.34 a B</td>
<td></td>
</tr>
<tr>
<td>Low&lt;sup&gt;d&lt;/sup&gt;</td>
<td>CTRL</td>
<td>2.10 a A</td>
<td>0.77 a b'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AW</td>
<td>2.09 a A</td>
<td>1.19 a b'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% LA</td>
<td>1.86 a A</td>
<td>0.62 a b'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of HA</td>
<td>1.77 a A</td>
<td>0.51 a b'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of PA</td>
<td>1.88 a A</td>
<td>0.84 a b'</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Swab samples were collected 5 min after antimicrobial spray treatment.

<sup>b</sup> Swab samples were collected prior to needle tenderization.

<sup>c</sup> Least-squares means within a sampling day and lacking common letters (a and b) differ (P < 0.05). Least-squares means in the same spray treatment and lacking common letters (a and b) differ (P < 0.05).

<sup>d</sup> Least-squares means within a sampling day with the same letter are not significantly different (P > 0.05). Least-squares means in the same spray treatment and lacking common letters (a' and b') differ (P < 0.05).

The cells of non-O157 STEC on high-inoculated subprimals on day 0 and after 14 days of vacuum storage at 4°C are presented in Table 2. No immediate effect due to spray treatment was observed (P = 0.102); however, surface pathogens on spray-treated samples were significantly (P = 0.0002) less than CTRL subprimals after storage. Specifically, the HA- and PA-treated subprimals had significant fewer cells compared with other treatments after 14 days of storage. The LA subprimals, with fewer cells than the CTRL samples, were similar (P = 0.62) to samples from the AW treatment group. Though the amount of reduction of non-O157 STEC was small (0.4 and 0.3 log CFU/50 cm²), LA or PA treatment at low-level inoculation on day 0 was significantly lower (P = 0.039) than the CTRL. After 14 days of storage, the LA and HA treatments had fewer (P = 0.011) surface non-O157 STEC strains than the CTRL. These results were in agreement with previous work (5) that LA combined with vacuum storage at refrigeration temperature effectively reduced individual strains of E. coli O26 and O111, separately. In addition, though not statistically significant, there was little pathogen reduction in CTRL and AW groups during storage. Similarly, Lemmons et al. (18) found that populations of STEC on beef surfaces were not greatly reduced on either untreated or AW-treated beef samples stored in a vacuum-packaged and refrigerated condition.

Overall, the results of this study showed that the effects of AW treatment on the reduction of surface E. coli O157:H7 on day 0 varied according to different inoculation levels and microorganisms. Wolf et al. (33) indicated that water spray treatment minimally reduced E. coli O157:H7 and non-O157 STEC on chilled beef surface, whereas Cutter and Rivera-Betancourt (5) found a substantial reduction of these organisms by water treatment through spray interventions. Additionally, the antimicrobial effect of water could be influenced by the time, pressure, and temperature at which it was applied (11).

Kalchayanand et al. (15) reported that antimicrobial treatments, such as LA and PA, used for E. coli O157:H7 were at least as effective for non-O157 STEC on prerigor beef; however, PA had a lower antimicrobial activity than LA. Furthermore, the effectiveness of PA varied in different studies. Penney et al. (22) reported substantial antimicrobial activity of PA against E. coli O157:H7 on the external carcass surfaces of prerigor beef inoculated with fecal contamination. However, other studies demonstrated less effectiveness of the PA treatment against coliforms or E. coli O157:H7 on either

**TABLE 2. Levels of non-O157 STEC recovered from beef subprimal surfaces immediately after antimicrobial spray treatment and after 14 days of storage at 4°C**

<table>
<thead>
<tr>
<th>Inoculum concn</th>
<th>Spray treatment</th>
<th>Log CFU/50 cm²</th>
<th>Day 0&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 14&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>High&lt;sup&gt;d&lt;/sup&gt;</td>
<td>CTRL</td>
<td>5.99 a A</td>
<td>5.44 a B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AW</td>
<td>5.70 a A</td>
<td>5.23 B b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% LA</td>
<td>5.47 a A</td>
<td>5.14 b b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of HA</td>
<td>5.75 a A</td>
<td>4.75 c b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of PA</td>
<td>5.68 a A</td>
<td>4.91 c b</td>
<td></td>
</tr>
<tr>
<td>Low&lt;sup&gt;d&lt;/sup&gt;</td>
<td>CTRL</td>
<td>2.30 a’ a’</td>
<td>1.31 a’ b’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AW</td>
<td>2.06 a’ b’ a’</td>
<td>1.59 a’ b’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% LA</td>
<td>1.81 b’ a’ b’</td>
<td>0.69 c’ b’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of HA</td>
<td>2.04 a’ b’ a’</td>
<td>0.64 c’ b’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 ppm of PA</td>
<td>1.98 b’ a’ b’</td>
<td>0.91 b’ c’ b’</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Swab samples were collected 5 min after antimicrobial spray treatment.

<sup>b</sup> Swab samples were collected prior to needle tenderization.

<sup>c</sup> Least-squares means within a sampling day and lacking common letters (a to c) differ (P < 0.05). Least-squares means in the same spray treatment and lacking common letters (a and b) differ (P < 0.05).

<sup>d</sup> Least-squares means within a sampling day and lacking common letters (a’ to c’) differ (P < 0.05). Least-squares means in the same spray treatment and lacking common letters (a’ and b’) differ (P < 0.05).
postrigror or prerigor beef carcass (9, 16). The inconsistent efficacy of PA could be due to the temperature of the meat and the PA solution, disparity of the meat sources, and the antimicrobial-resistant strains used in these studies. Our data indicate that small but significant reductions of non-O157 STEC on postrigror beef could be obtained by using PA. The antimicrobial effect of PA could be varied due to different states of meat (prerigror versus postrigror), different harvest parts, and surface contamination levels.

The studies regarding the efficacy of HA against either E. coli O157:H7 or non-O157 STEC are limited due to its recent acceptance as an antimicrobial agent on meat. No research has been found regarding the effectiveness of HA on the reduction of foodborne pathogens on postrigror beef. However, previous studies demonstrated the application of HA at a concentration of 200 ppm reduced E. coli O157:H7 on both prerigror beef and hide-on carcass by approximately 2 log CFU/cm² for both sites (14, 27). In the current study, HA treatment resulted in less than a 1-log CFU/50-cm² reduction for either E. coli O157:H7 or non-O157 STEC at both high and low inoculation levels on beef surfaces before storage. The antimicrobial activity of HA may be affected by the temperature and characteristics of the meat surface at which it was applied.

**Bacterial translocation and thermal inactivation.**

On day 14, subprimals from each treatment group were blade tenderized and portioned into retail-ready steaks. Assessment of surface pathogen translocation was performed by the collection of steak surface swabs prior to transportation and cooking. Approximately 2 log CFU/50 cm² of E. coli O157:H7 were recovered on the surface of steaks derived from high-level-inoculated, blade-tenderized subprimals (data not shown). Furthermore, the data imply that spray interventions applied to the surface of subprimals prior to vacuum-packaged storage had no influence (P = 0.894) on the internalization of pathogens following blade tenderization and steak portioning. Similarly, approximately 3 log CFU/50 cm² (data not shown) of non-O157 STEC were on the surfaces of raw steaks obtained from high-inoculated subprimals after 14 days of vacuum storage and mechanical tenderization. As with the E. coli O157:H7 trial group, subprimal spray intervention treatment had no influence (P = 0.446) on the population of pathogens on the steak surface.

The translocation of pathogens into the internal portions of consumer steaks portion through mechanical tenderization has been documented (6) and is of concern, because the internalized pathogens have the potential to survive exposure to previously considered lethal cooking temperatures. Table 3 illustrates the numbers of internal core samples, raw (noncooked) and grilled (50 or 70 °C), which were positive for E. coli O157:H7. In samples derived from the high-level-inoculated subprimals, 100% (n = 9) of uncooked cores were positive for E. coli O157:H7. Over 30% (n = 3) of samples obtained from steaks cooked to 50°C were positive for E. coli O157:H7, regardless of treatment. In samples obtained from steaks cooked to 70°C, the LA-treated subprimals completely eliminated detectable E. coli O157:H7 cells; however, more than 40% (n = 4) of the samples from the CTRL, AW, and HA treatment groups were E. coli O157:H7 positive. At low inoculation, the percentage of positive core samples was lower than that of the high-inoculated steaks. These results are important, as the low-level inoculation more appropriately represents the possible level of contamination within the industry. Nonetheless, E. coli O157:H7 was detected in 56% (n = 5, PA) to 78% (n = 7, CTRL and AW) of raw steak cores. As expected, after cooking, a marked decline in positive samples was observed. At an internal temperature of 50°C, a range from 22% (n = 2, CTRL and LA) to 33% (n = 3, AW and HA) of samples were positive for E. coli O157:H7, while steak cores from subprimals treated with PA were negative. After cooking to 70°C, E. coli O157:H7 was nondetectable in core samples from LA- and PA-treated subprimals. However, the pathogen was detected in 22% (n = 2), 22% (n = 2), and 11% (n = 1) of core samples from the CTRL, AW, and HA treatments, respectively.

The influence of cooking temperature and spray treatment on the detection of non-O157 STEC in low-inoculated steak cores is shown in Table 4. One hundred percent of raw samples (n = 9) from the CTRL, AW, LA, and HA groups were positive for non-O157 STEC prior to cooking, while PA-treated samples were 89% (n = 8) positive. Similar to O157:H7 results, cooking resulted in a noticeable reduction of the proportion of steaks positive for the pathogen. When cooked to 50°C, the percentage of positive samples for the CTRL, AW, LA, HA, and PA treatments showed varied proportions of positive cores with 44% (n = 4), 77.8% (n = 7), 22.2% (n = 2), 22.2% (n =

### Table 3. Detection of E. coli O157:H7 in the tenderized steaks, noncooked (NC) and cooked to internal temperatures of 50 and 70°C

<table>
<thead>
<tr>
<th>Inoculum concn</th>
<th>Temp (°C)</th>
<th>CTRL</th>
<th>AW</th>
<th>5% LA</th>
<th>200 ppm of HA</th>
<th>200 ppm of PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>NC</td>
<td>9 A</td>
<td>9 A</td>
<td>9 A</td>
<td>9 A</td>
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<tr>
<td></td>
<td>50</td>
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<td>5 A</td>
<td>6 A</td>
<td>0 A</td>
<td>4 A</td>
<td>2 A</td>
</tr>
<tr>
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<td>5 A</td>
</tr>
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<td>0 A</td>
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<tr>
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<td>2 A</td>
<td>2 A</td>
<td>0 A</td>
<td>1 A</td>
<td>0 A</td>
</tr>
</tbody>
</table>

a Values in each cooking temperature with the same letter are not significantly different (P > 0.05).
2), and 11.1% (n = 1) positive, respectively. Compared with O157:H7 results, a similar trend was observed, with the most survivors remaining on the AW-treated samples and the fewest from the 200 ppm of PA treatments. Treatments with HA did not differ significantly from the CTRL. Interestingly, when cooked to 70°C, only core samples from CTRL steaks indicated a greater reduction due to a higher cooking temperature. Samples from the AW group did not change; however, LA, HA, and PA treatments had a higher percentage of positive samples than the CTRL, with 44% (n = 4), 56% (n = 5), and 33% (n = 3), respectively, but why some treatments contained higher positive samples after cooking to 70°C was not clear.

Our study stands in agreement with previously published results. Luchansky et al. (19) found that cooking to a medium degree of doneness (71°C) did not entirely inactivate interior E. coli O157:H7 or non-O157 STEC in tenderized beef steaks. They also hypothesized that the uneven heating of the tenderized samples due to tissue rupture caused by a tenderization process could be the major factor for compromising bacterial thermal inactivation (20). Other factors, such as fat deposition, steak thickness, and types of cooking, may also affect the efficiency of a cooking process (1, 28).

In this study, by using a real-time PCR method, the data of the low-inoculated core samples after cooking to either internal temperature (50 or 70°C) indicated that the strain of serogroup O103 was likely to be more heat resistant than the others, while the strain of serogroup O111 was likely to be more heat sensitive (data not shown). Others have also indicated that serogroup O103 in an inoculated liquid food product was more heat resistant than other serogroups of non-O157 STEC (8). Thus, the efficacy of thermal inactivation for non-O157 STEC strains were likely to be different from one to another due to a heterogeneous family of non-O157 STEC.

The present work mimics the safety of the steaks after cooking in a restaurant. Although postharvest interventions, such as antimicrobial spray, are applied on beef subprimals to decrease levels of pathogens before they go into market, this study shows that a temperature of 70°C (medium degree of doneness) used for cooking needle-tenderized steaks may not be able to eliminate internalized E. coli O157:H7 or STEC, and a higher degree of doneness for the tenderized beef is recommended to guarantee no survivors during cooking, as demonstrated in this study. Additionally, the findings of this study show that E. coli O157:H7 and non-O157 STEC may not respond similarly when in contact with antimicrobial spray, showing different susceptibilities to this intervention. Therefore, antimicrobial interventions currently used for E. coli O157:H7 should be reevaluated and validated to each specific STEC.

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**REFERENCES**