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

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

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

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

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

See next page for additional authors

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Efficacy of Antimicrobial Compounds on Surface Decontamination of Seven Shiga Toxin–Producing *Escherichia coli* and *Salmonella* Inoculated onto Fresh Beef†

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

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, State Spur 18D, Clay Center, Nebraska 68933-0166, USA

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ABSTRACT

Several antimicrobial compounds have been used in commercial meat processing plants for decontamination of pathogens on beef carcasses, but there are many commercially available, novel antimicrobial compounds that may be more effective and suitable for use in beef processing pathogen-reduction programs. Sixty-four prerigor beef flanks (cutaneous trunci) were used in a study to determine whether hypobromous acid, neutral acidified sodium chlorite, and two citric acid–based antimicrobial compounds effectively reduce seven Shiga toxin–producing *Escherichia coli* (STEC) serogroups and *Salmonella* on the surface of fresh beef. Two cocktail mixtures were inoculated onto prerigor beef flank surfaces. Cocktail mixture 1 was composed of STEC serogroups O26, O103, O111, O145, and O157; and cocktail mixture 2 was composed of STEC serogroups O45, O121, and O157 and *Salmonella*. The inoculated fresh beef flanks were subjected to spray treatments with four antimicrobial compounds. Following antimicrobial treatments, both control and treated fresh beef samples were either enumerated immediately or were stored for 48 h at 4°C before enumeration. All four antimicrobial compounds caused 0.7- to 2.0-log reductions of STEC, *Salmonella,* aerobic plate counts, and *Enterobacteriaceae*. Results also indicated that the four antimicrobial compounds were as effective at reducing the six non-O157 STEC strains as they were at reducing *E. coli* O157:H7 on the surfaces of fresh beef. The recovery of all seven STEC strains and *Salmonella* in a low-inoculation study indicated that none of the four antimicrobial compounds eliminated all of the tested pathogens.

Animal products may be contaminated with foodborne pathogens and can support their growth if not properly handled, processed, cooked, and preserved, which may pose a significant public health threat. If a contaminated product makes it into commerce in today's interconnected global economy, risk of a widespread foodborne outbreak is needlessly high. The result could be lost domestic and international sales, a damaged brand reputation, and even a hit to the U.S. economy from lost trade and employment. *Escherichia coli* O157:H7 and *Salmonella* are common human infectious agents worldwide *(17, 29)*. Both *Salmonella* and Shiga toxin–producing *E. coli* (STEC) have been found to contaminate carcasses at commercial beef processing facilities *(3, 5, 8)*. *Salmonella* prevalence on hides and on carcasses pre- and postintervention averaged 89.6, 50.2, and 0.8%, respectively *(8)*. Bosilevac et al. *(7)* analyzed 4,136 ground beef samples collected from seven regions of the United States and reported an overall 4.2% prevalence rate of *Salmonella* strains. *Salmonella enterica* serovars Typhimurium and Newport are commonly identified in clinical samples and, in 2003, represented 11 and 30%, respectively, of the reported isolates associated with beef *(12)*. *Salmonella* Typhimurium DT-104 caused an outbreak in the northeastern United States from August 2003 to January 2004 that was linked to commercial ground beef *(14)*.

In addition to *E. coli* O157:H7, the Centers for Disease Control and Prevention (CDC) has identified six serogroups as the cause of 71% of non-O157 STEC infection in the United States *(9)*. These pathogens also are associated with cattle; the reported prevalence of non-O157 STEC on beef cattle hides and in feces, between 7 and 56.6% *(3, 19, 42)*, represents a potential source of beef carcass contamination *(27)*. The rate of contamination of beef products with non-O157 STEC strains is probably the same or similar to that of *E. coli* O157:H7. Barkocy-Gallagher et al. *(3)* 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 STEC strains (8%) was reported on carcasses after the application of multiple-hurdle interventions *(2)*. Bosilevac et al. *(6)* recently reported that the prevalence of non-O157 STEC strains

* Author for correspondence. Tel: 402-762-4224; Fax: 402-762-4149; E-mail: norasak.kalchayanand@ars.usda.gov.

† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

from the imported and domestic boneless beef trim used for ground beef in the United States was as high as 10 to 30%.

STEC and *Salmonella* threaten consumers' health and cause economic loss due to illnesses, product condemnation, and lower product demand. The desired outcome, of course, is to prevent foodborne outbreaks caused by contamination of the red meat supply. Carcass decontamination interventions contribute to the production of carcasses with lower levels of bacteria and reduced incidence of enteric pathogens, which helps meet regulatory requirements during slaughter. Common antimicrobial interventions, including thermal treatments and chemical solutions, have been used to successfully decontaminate meat and meat product surfaces from *E. coli* O157:H7 and *Salmonella* during the harvesting process *(10, 16, 18, 21, 22, 26, 34, 37).* These interventions have also effectively reduced non-O157 STEC strains on surfaces of fresh beef *(23).* Numerous other commercially available chemical compounds, such as hypobromous acid, neutral acidified sodium chlorite, and citric acid–based antimicrobial agents, have been developed that may possess desirable characteristics and may cost less. There is limited information on the efficacy of these compounds against O157:H7 STEC, non-O157 STEC strains, and *Salmonella,* and their efficacy when applied on the surface of fresh beef needs to be determined. In this study, hypobromous acid, neutral acidified sodium chlorite, and citric acid–based antimicrobial agents were evaluated for their effectiveness in reducing seven serotypes of STEC and *Salmonella* inoculated onto fresh beef.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation of inocula. Strains of non-O157 *E. coli* serotypes O26 (:H11, 3891 and :H11, 3392; both human isolates), O45 (:H2, 01E-1269; human isolate), O45 WDG3 (isolated from cattle hide), O103 (:H2, 2421; human isolate), O111 (:NM, 1665 and :NM, ECRC 3007:85; both human isolates), O121 (:H19, O1E-2074; human isolate), O121:H7 (isolated from ground beef), and O145 (:NM, GS5578620; human isolate and a ground beef isolate); *E. coli* O157:H7 (ATCC 43895 and FSIS #4); *Salmonella* Newport (13109 and 15124; isolated from beef carcass); and *Salmonella* Typhimurium (14218 and DT-104) from the U.S. Meat Animal Research Center (USMARC) culture collection were grown for 16 to 18 h at 37°C in nutrient broth (BD, Sparks, MD). The cultures were adjusted to an optical density that was equivalent to a population of approximately 1.5×10^8 CFU/ml, using a spectrophotometer at 600 nm. Two inocula were used in this study because serogroups O45 and O103 have similar morphology, which makes them difficult to separate from each other during enumeration and detection. Inoculum 1 consisted of an equal volume of each strain of O26, O103, O111, O145, and O157 to form a nine-strain cocktail mixture. Inoculum 2 consisted of an equal volume of each strain of O45, O121, O157, and *Salmonella* to form a 10-strain mixture. These two cocktails were each diluted to approximately 1.5×10^7 or 1.5×10^4 CFU/ml for high and low inoculations, respectively. Inoculum 1 was diluted in maximum recovery diluent (BD), whereas inoculum 2 was diluted with purge to provide a typical background flora. The fresh beef flanks used for this inoculation study initially had low *Enterobacteriaceae* counts (EBC; <20 CFU/cm^2) that could be detected after treatments. EBC has been used in the beef industry as an indicator for pathogens during processing steps. Therefore, purge was aseptically collected from vacuum-packaged beef subprimals that had been stored at −20°C and then thawed at 4°C. The average initial population (aerobic plate count [APC] and EBC) of beef purge was approximately 2 to 3×10^3 CFU/ml. The inocula were then placed in an ice bath while each day's samples were processed (2 h) to restrict further cell growth before use.

Fresh beef inoculation. Prerigor beef flanks (cutaneous trunci muscle; 16 flanks per treatment) were collected from a local beef cattle processing plant (8 flanks for inoculum 1 and 8 flanks for inoculum 2) within 25 min postexsanguination and were transported to the USMARC laboratory within 2 h in insulated containers for use in this study. The majority of the surface area of beef flanks used was lean tissue, with some parts covered with adipose tissue. The average surface pH of the prerigor beef flanks was 6.8. One intervention treatment × inoculum combination was processed per day. Each day 16 flanks were divided into two groups of eight flanks. The first group was inoculated with high levels of inoculum, and the second group was inoculated with low levels of inoculum. 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. An aliquot of 50 μl of either inoculum was inoculated on individual 25-cm^2 sections, spread over the area, and left to stand for 15 min at room temperature to allow bacterial cells to attach before the flanks were subjected to antimicrobial treatments. The final cell concentration for high and low levels of inoculum was approximately 3×10^4 and 3×10^1 CFU/cm^2, respectively.

Antimicrobial treatments and sample collections. The antimicrobial compounds used in this project are generally recognized as safe, and the applied concentrations were within the recommended range. The following antimicrobial treatments were applied (22 to 25°C) to the inoculated fresh beef: (i) hypobromous acid (300 ppm, pH = 6.7; prepared from hydrobromic acid as per manufacturer's recommendation; Environ Tech Chemical Services, Modesto, CA), (ii) neutral acidified sodium chlorite (1,000 ppm of sodium chlorite and 24 ppm of chlorine dioxide, pH = 6.5; prepared using Olas TI on-line activation systems as per manufacturer's recommendation; Dan Mar Co., Arlington, TX), (iii) CAB-1 (2% Citrilow, pH = 1.8; Safe Food Corp., North Little Rock, AR), and (iv) CAB-2 (1:50 FreshFx, pH = 1.8; SteriFX Inc., Shreveport, LA). The antimicrobial treatments were performed using a model spray wash cabinet with three oscillating flat-pattern spray nozzles (SS5010, Spray Systems Co., Wheaton, IL) at 60 cycles per min with a flow rate of 5.7 liters/min. The distance between the nozzles and the surface of the beef flanks was 17 cm. Antimicrobial treatments were sprayed at 20 lb/in^2 for 15 s; after the excess liquid was allowed to drip off for 30 s, the surface pH of treated fresh beef was 6.2, 6.2, 3.7, and 3.6 for hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2, respectively. Before the beef flanks were treated with antimicrobials, four 25-cm^2 tissue sections were randomly excised and placed individually into four filtered bags (Whirl-Pak, Nasco, Ft. Atkinson, WI) to serve as pretreatment controls. After the treatments, an additional four 25-cm^2 tissue sections were excised and individually placed in another four filtered bags. Samples were enumerated within 10 min following each treatment. A second set of eight samples (four untreated control and four treated) were stored aerobically in filtered bags for 48 h at 2 to 4°C before enumeration to determine whether any of the antimicrobial treatments had residual effects during chilling. The surface pH values of control and treated fresh

beef samples after 48 h at 4°C were 5.8, 5.8, 5.8, 5.6, and 5.5 for no treatment, hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2 treatment, respectively.

Microbiological analyses. Control and treated tissue samples (25-cm^2 sections) were neutralized by adding 50 ml of Dey-Engley broth (BD) supplemented with 0.3% soytone and 0.25% sodium chloride and were homogenized for 1 min using a stomacher (BagMixer 400, Interscience, Weymouth, MA). An aliquot (1 ml) of each sample was transferred into a 2-ml cluster tube and was serially 10-fold diluted with maximum recovery diluent (BD). Appropriate dilutions of control samples (initial population) and treated samples were spiral plated on differential USMARC chromogenic medium *(24)*. Aerobic bacteria and *Enterobacteriaceae* were plated per manufacturer's recommendation on Petrifilm EBC plates (3M, St. Paul, MN) and on nonselective medium for APC using Petrifilm (3M). The USMARC chromogenic agar medium was formulated based on the composition of MacConkey medium, but without lactose and neutral red. The basal formulation of the selective differential medium was as follows: 17.0 g/liter Bacto Peptone (BD), 3.0 g/liter Proteose Peptone (BD), 5.0 g/liter sodium chloride (Sigma, St. Louis, MO), 1.0 mg/liter crystal violet (Sigma), 3 g/liter bile salts no. 3 (BD), 6.0 g/liter L-sorbose (Sigma), 6.0 g/liter D-raffinose (Sigma), 20 mg/liter phenol red (Sigma), 1.5 mg/liter bromothymol blue (Sigma), and 15 g/liter Bacto agar (BD). The chromogenic plates were incubated at 37°C for 24 h and then at room temperature for 30 min for full color development for enumeration. The colony colors developed on the USMARC chromogenic agar medium were turquoise blue, blue-green, light green, dark blue green, light blue gray, purple, and green for O26, O45, O103, O111, O121, O145, and O157, respectively. Petrifilm plates were incubated according to the manufacturer's recommendation. The limit of detection using a spiral plater (Spiral Biotech, Norwood, MA) was 60 CFU/cm^2. CFUs from untreated control and treated samples were counted from Petrifilm and from USMARC chromogenic agar plates. Colony colors representing each STEC serogroup were counted, and up to 20 presumptive colonies from plates of non-O157, O157 STEC, and *Salmonella* (colorless with pink halo) were picked for confirmation. STEC strains were confirmed using multiplex PCR *(32)*. A separate multiplex PCR *(25)* was used to confirm *Salmonella*.

For low-level inoculation, both control and treated samples were enriched at 25°C for 2 h and at 42°C for 6 h and then were held at 4°C before immunomagnetic separation of target organisms.

A 1-ml aliquot of each enriched sample inoculated with inoculum 1 was added to 25-μl mixtures of anti-O26, -O103, -O111, -O145, and -O157 immunomagnetic beads (Dynabeads, Invitrogen, Carlsbad, CA), and each enriched sample inoculated with inoculum 2 was added to 20 μl of anti-O157 immunomagnetic beads (Dynabeads). Each enriched sample containing immunomagnetic beads was subjected to immunomagnetic separation as described previously *(3, 31)*. The bacterial bead complexes were spread plated on CHROMAgar O157 (DRG International, Mountainside, NJ), supplemented with 5 mg of novobiocin/liter and 1.0 mg of potassium tellurite/liter for *E. coli* O157:H7, and on USMARC chromogenic agar for non-O157 STEC strains. After immunomagnetic separation for STEC, each enriched sample was streaked for isolation for *Salmonella* using xylose lysine deoxycholate agar (BD) and was streaked on the USMARC chromogenic agar *(24)* for STEC serogroups O45 and O121. 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 for STEC strains *(32)* and for *Salmonella (25)*.

Statistical analyses. Bacterial populations of untreated control and treated samples were transformed to log CFU per square centimeter values from four experimental replications of each treatment × eight tissue sections ($n = 32$). One-way analysis of variance 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 $P \leq 0.05$. Percentage of recovery for each organism was calculated by dividing the numbers of bacteria of the positive treated sample by the numbers of bacteria of the positive untreated sample and multiplying by 100; percentages of recovery were compared among organisms within treatments using WINPEPI Compare2 *(1)*.

RESULTS AND DISCUSSION

Several studies have shown that most contamination of fecal origin occurs during hide and skin removal and evisceration processes *(4, 27, 38)* and that it is best to decontaminate meat immediately, before bacteria attach firmly to its surface. U.S. Department of Agriculture, Food Safety and Inspection Service policy calls for a decontamination step as part of the slaughtering and dressing process *(44)*. In this study, four antimicrobial compounds were selected based on their pH and commercial availability. Hypobromous acid and neutral acidified sodium chlorite solutions have neutral pH, whereas CAB-1 and CAB-2 solutions have acidic pH. The mean log reductions from hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2 treatments are presented in Table 1. In general, all four antimicrobial compounds immediately reduced ($P \leq 0.05$) the populations of O26, O45, O103, O111, O121, O145, *E. coli* O157:H7, *Salmonella,* APC, and EBC on fresh beef, ranging from 0.7 to 2.0 log CFU/cm^2.

The effectiveness of the four antimicrobial compounds depended on the target organisms (Table 1). Spray treatments with hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2 resulted in a 1.0- to 1.7-log reduction of serogroups O26 and O45, and all four compounds were equally effective at inactivation ($P > 0.05$). Hypobromous acid and neutral acidified sodium chlorite were less effective in reducing *Salmonella* compared with CAB-1 and CAB-2. There was no difference ($P < 0.05$) in reduction of serogroups O103, O111, O145, and O157 among hypobromous acid, neutral acidified sodium chlorite, and CAB-2. However, CAB-2 was more effective than hypobromous acid and neutral acidified sodium chlorite at reducing *Salmonella* ($P < 0.05$). CAB-1 was more effective ($P < 0.05$) than any of the other treatments at reducing populations of O103, O111, and O145 on fresh beef surfaces. For other organisms, the comparative efficacy of CAB-1 versus other treatments depended on the target organism.

Hydrobromous acid is widely used as a disinfectant for water treatment and for treating industrial or commercial water-cooling systems *(35, 39)*; it was previously shown to effectively reduce the population of *E. coli* O157:H7 and

TABLE 1. *Reduction of pathogenic bacteria following treatment with antimicrobial compounds*[a]

	Reduction of pathogens (log CFU/cm^2)											
	Inoculum 1						Inoculum 2					
Treatment[b]	O26	O103	O111	O145	O157	APC	O45	O121	O157	Sal	APC	EBC
HOBr	1.0 BC x[c]	1.3 B x	1.0 BC x	1.1 B x	1.0 B x	1.1 B	1.1 B x	1.3 AB x	1.0 BD x	0.8 D x	0.8 B	0.8 C
HOBr chilled	1.3 BCD x	1.3 B x	1.2 BC x	1.4 BC x	1.2 B x	1.1 B	1.2 B x	1.7 A x	1.4 BC x	1.2 BC x	1.2 B	1.1 BC
nASC	1.3 BCD x	1.1 B x	1.2 BC x	1.0 B x	1.0 B x	1.2 B	1.0 B x	0.9 B x	1.0 BD x	0.7 D x	1.1 B	1.1 BC
nACS chilled	2.0 AD x	1.9 A x	2.0 A x	2.1 A x	1.9 A x	2.0 A	1.6 A x	1.9 A x	1.8 A x	1.2 BC y	1.5 A	1.7 A
CAB-1	1.7 AC x	2.0 A x	1.9 A x	1.6 ACD x	1.7 AC x	1.8 A	1.4 AB y	1.8 A x	1.5 AC xy	1.7 A xy	1.6 A	1.5 AB
CAB-1 chilled	1.7 AC x	1.9 A x	1.9 A x	1.8 ACD x	1.7 AC x	1.7 A	1.5 A y	1.9 A x	1.6 AC y	1.6 A y	1.7 A	1.6 A
CAB-2	1.7 AC x	1.1 B yz	0.7 BC yz	1.1 B yz	1.3 B z	1.0 B	1.4 AB x	1.3 AB x	1.3 BC x	1.4 AC x	1.4 A	1.6 A
CAB-2 chilled	2.3 A x	1.8 A x	1.7 A x	1.8 ACD x	1.2 BC y	1.7 A	1.6 A x	1.4 A x	1.5 AC x	1.6 A x	1.7 A	2.0 A

[a] The reductions were calculated as the difference between the initial population of untreated tissue samples (control) and the population from the treated tissue samples. APC, aerobic plate count; EBC, *Enterobacteriaceae* count.

[b] For each treatment, $n = 32$. HOBr, hydrobromous acid (300 ppm); nASC, neutral acidified sodium chlorite (1,000 ppm); CAB-1, Citrilow (2%); CAB-2, FreshFX (2%).

[c] Mean comparisons were performed independently for each inoculum. Within a treatment type, means with no common lowercase letter (x through z) in the same row of each inoculum are significantly different ($P \leq 0.05$). For each inoculum, within a serogroup, means with no common uppercase letter (A through D) are significantly different ($P \leq 0.05$).

Salmonella on the surfaces of fresh beef and beef hearts *(21)*. In contrast, when beef trimmings or cheek meat tissues were dipped into 225- or 300-ppm hypobromous acid solution, populations of STEC, including non-O157 STEC and *Salmonella,* were not reduced *(15, 36)*. Methods of applying hypobromous acid solutions, therefore, need more investigation.

According to the manufacturer's recommendation, the neutral acidified sodium chlorite solution was activated with phosphoric acid to pH 2.2 to 3.0 and then was further diluted with a small amount of sodium chlorite to elevate the pH to 6.5, such that the resultant concentration of biocide did not exceed 1,200 ppm. When neutral acidified sodium chlorite solution was used as a spray treatment, reductions of the seven serogroups of STEC and *Salmonella* were similar to the reductions from treatment with hypobromous acid. Few studies have examined the efficacy of neutral acidified sodium chlorite. However, there have been reports of reductions of *Salmonella* and *E. coli* O157:H7 on beef carcass tissue after washing or spray treatment with sodium chlorite activated (acidified) with citric or phosphoric acid *(33, 45)* or a combination of a water wash followed by an acidified sodium chlorite spray *(11)*.

CAB-1 and CAB-2 were the two acidic antimicrobial solutions used in this study; both are a blend of citric and inorganic acids. CAB-1 is a combination of citric and hydrochloric acids, whereas CAB-2 is a combination of citric, hydrochloric, and phosphoric acids. In the meat industry, lactic and acetic acid solutions are the most commonly used organic acids and have been shown to effectively reduce *E. coli* O157:H7 *(33)* as well as non-O157 STEC on surfaces of fresh beef *(15, 23)*. There is limited information on the efficacy of citric acid–based antimicrobial compounds against pathogens on fresh meat. In this study, spray treatment with CAB-1 (2%, pH 1.8) and CAB-2 (2%, pH 1.8) immediately resulted in 0.7- to 2.0-log CFU/cm^2 reductions ($P \leq 0.05$) in the population of STEC serogroups O26, O45, O103, O111, O121, O145, and O157 and *Salmonella* on the surface of beef flanks. Similar reductions of *E. coli* O157:H7 were reported for beef heads and cheek meat treated with 2% CAB-2 *(22)*. CAB-1 and CAB-2 both contain citric acid as a main ingredient, and Tamblyn and Conner *(40)* reported that citric acid (4%) resulted in 1.9-log reduction of *Salmonella* Typhimurium on chicken skin. Reduction of *E. coli* O157:H7 on lean and adipose tissues of beef carcasses treated with different concentrations of citric acid was also reported by Cutter and Siragusa *(13)*.

Fresh beef samples (control and treated samples) were chilled for 48 h at 4°C before enumeration to simulate carcass processing and to determine the population on treated fresh beef before further fabrication. In fresh beef tissues sprayed with hypobromous acid and CAB-1 and chilled for 48 h at 4°C, no further reduction ($P > 0.05$) of all seven STEC, APC, EBC, and *Salmonella* was found, with the exception of *Salmonella* treated with hypobromous acid. In contrast, on fresh beef surfaces sprayed with neutral acidified sodium chlorite and CAB-2 and chilled at 4°C for 48 h, bacterial populations were further reduced. Additional reductions were observed for serogroups O45, O103, O111, O145, and O157 and for APC, EBC, and *Salmonella* after spray treatment with neutral acidified sodium chlorite and chilling for 48 h at 4°C. Spray treatment with CAB-2 and chilling at 4°C for 48 h caused further reductions only in populations of serogroups O103, O111, and O145. Comparison of the treatments before and after chilling shows that neutral acidified sodium chlorite might have a residual effect on the pathogens, with the exception of serogroup O26, whereas CAB-2 might have a residual effect only on serogroups O103, O111, and O145. Normally, chilling slows the growth of most bacteria, and temperatures just above the freezing point can kill or injure bacterial cells *(20)*. Conventional chilling can reduce the microbial populations on carcasses by 0.3 to 0.7 log *(30, 41)*. Chilling

may have increased the degree of injury of certain pathogens already damaged by the antimicrobial treatments; and, thus, they could not be enumerated well on selective medium. The residual effect of neutral acidified sodium chlorite and CAB-2 in combination with chilling on bacterial growth, particularly of pathogens, needs to be further investigated, but it is beyond the scope of the present study.

Numerous antimicrobial interventions targeting *E. coli* O157:H7 have been developed and implemented to successfully decontaminate meat and meat products during the harvesting process. In this study, *E. coli* O157:H7 was included in the inocula, along with six serogroups of non-O157 STEC and *Salmonella*. Although hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2 were generally able to reduce populations of STEC, it is important to determine the relative effectiveness of antimicrobial compounds for reducing non-O157 STEC and *Salmonella* compared with *E. coli* O157:H7. Table 1 shows the effectiveness of each antimicrobial compound in reducing the populations of the six serogroups of non-O157 STEC inoculated onto fresh beef flanks. Overall, on fresh beef treated with these compounds, there were 0.7- to 2.0-log CFU/cm^2 reductions of APC, EBC, STEC serogroups O26, O45, O103, O111, O121, O145, O157:H7, and *Salmonella*. Hypobromous acid caused reductions of 1.0 to 1.3 and 0.8 log CFU/cm^2 of the six non-O157 STEC strains and *Salmonella,* respectively; these reductions were not different ($P > 0.05$) from that of *E. coli* O157:H7. Hypobromous acid has been reported to cause reductions of 1.6 to 2.1 and 0.7 to 2.3 log CFU/cm^2 of *E. coli* O157:H7 and *Salmonella,* respectively, on surfaces of fresh beef and beef hearts *(21)*. Neutral acidified sodium chlorite reduced non-O157 STEC and *Salmonella* ranging from 0.9 to 1.3 and 0.7 log CFU/cm^2, respectively. The reductions of six non-O157 STEC strains and *Salmonella* were similar ($P > 0.05$) to the reduction of *E. coli* O157:H7 after neutral acidified sodium chlorite treatment. This is similar to the results of treatment with sodium chlorite activated with sodium hydrogen sulfate to pH 2.5, which caused 0.6- to 2.0-log CFU/cm^2 reductions in the top six non-O157 STEC strains inoculated on surfaces of fresh beef *(23)*. Another study demonstrated that a 1.9- to 2.3-log reduction of *Salmonella* and *E. coli* O157:H7 on beef carcass tissue can be achieved using sodium chlorite activated with citric acid *(33)*. Castillo et al. *(11)* also reported that a water wash followed by acidified sodium chlorite treatment reduced *E. coli* O157:H7 and *Salmonella* by up to 4.6 log.

CAB-1 and CAB-2 (acidic antimicrobial compounds) reduced ($P \leq 0.05$) APC, EBC, non-O157 STEC, *E. coli* O157:H7, and *Salmonella* (Table 1). CAB-1 treatment caused a 1.4- to 2.0-log CFU/cm^2 reduction of six non-O157 STEC strains and *E. coli* O157:H7 on inoculated fresh beef. In a previous study, a 20-s spray application of the commercial antimicrobial compound Beefxide (a blend of citric and lactic acids) reduced populations of *E. coli* O157:H7 and *Salmonella* by 1.4 and 1.1 log CFU/100 cm^2, respectively, on inoculated fresh beef *(28)*. FreshFx caused 0.7- to 1.7-log CFU/cm^2 reductions of non-O157 STEC strains and *E. coli* O157:H7, as well as of *Salmonella,* on inoculated fresh beef. Kalchayanand et al. *(22)* demonstrated that a spray wash using 2% FreshFx generated a 1.1-log reduction of *E. coli* O157:H7 on beef head and cheek meat. The effectiveness of CAB-1 and CAB-2 in reducing *Salmonella* and non-O157 STEC strains ($P < 0.05$) was similar to the reduction of *E. coli* O157:H7 (Table 1). Several antimicrobial compounds commonly used in the beef industry also were reported to reduce the six non-O157 STEC strains on inoculated fresh beef as effectively as they reduced *E. coli* O157:H7 *(15, 23)*. The four studied antimicrobial compounds reduced APC by 0.8 to 1.8 log CFU/cm^2 and EBC by 1.2 to 2.0 log CFU/cm^2 after chilling at 4°C for 48 h. In a preliminary study, water (22 to 25°C) sprayed for 15 s at 20 lb/in^2 reduced both APC and EBC by 0.2 log CFU/cm^2 (data not shown); also, data from three commercial beef processing plants *(4)* indicated that water spraying has a minimal effect on reducing bacteria attached on the surface of fresh beef.

On the pathogens tested, hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2 treatments reduced STEC, *Salmonella,* APC, and EBC by 0.7 to 2.0 log CFU/cm^2 (Table 1). For an intervention to be considered effective, it should provide a reduction of at least 1 log cycle. This is based on the nationwide microbiological baseline study from 1992 to 1996 *(43),* which detected *E. coli* O157:H7 and *Salmonella* on 0.2 and 1.0% of carcasses, respectively; the actual populations of the pathogens on the positive carcasses from these studies showed that they were as low as 60 and 10 most probable number per 100 cm^2, respectively. Therefore, a decontamination intervention that achieves at least a 1-log reduction (90% inactivation) would probably reduce the number of carcasses testing positive for the pathogens by a similar amount. Under the study conditions, all four compounds tested could be used as effective interventions to reduce pathogens on surfaces of fresh beef if 1-log reduction is the minimum criterion (Table 1).

For the low-inoculation study, the low level of organisms could not be enumerated due to the limit of detection. Both control and treated samples were enriched and subjected to immunomagnetic separation before streaking for isolation; the recovery rates of treated samples after chilling for 48 h at 4°C are presented in Table 2. The recovery rate of STEC serogroups O26, O45, O103, O111, O121, O145, *E. coli* O157:H7, and *Salmonella* ranged from 14 to 100%, indicating that the degree of killing and sublethal injury depends on the bacterial strains and type of antimicrobial intervention used. Based on reduction data for the chilled beef samples (Table 1), the recovery rates generally corresponded to the enumerable levels. For example, there was 100% recovery of *Salmonella* after treatment with hypobromous acid and neutral acidified sodium chlorite, but lower recovery ($P < 0.05$) resulted after treatment with CAB-1 and CAB-2 (72 and 81%, respectively). CAB-1 and CAB-2 treatments led to greater reductions ($P < 0.05$) of *Salmonella* populations on fresh beef surfaces compared with treatments with hypobromous acid and neutral acidified sodium chlorite (Table 1). The

TABLE 2. *Recovery of STEC strains after antimicrobial interventions and after chilling for 48 h at 4°C*[a]

	% recovery relative to untreated control								
	Inoculum 1					Inoculum 2			
Treatment[b]	O26	O103	O111	O145	O157	O45	O121	O157	*Salmonella*
HOBr	71 A[c]	68 A	57 A	74 A	91 A	89 A	19 B	77 A	100 A
nASC	25 B	25 B	25 B	50 B	34 B	50 B	41 AB	31 B	100 A
CAB-1	16 B	45 AB	22 B	80 A	40 B	74 AB	62 A	50 AB	72 B
CAB-2	94 A	54 AB	44 AB	78 A	97 A	62 AB	14 B	60 A	81 B

[a] The percentage of recovery of each organism was calculated as the numbers of bacteria of the positive treated sample divided by the numbers of bacteria of the positive untreated control sample × 100. The untreated control was inoculated and sampled without being subjected to any treatments; recovery rates ranged from 75 to 100%.

[b] For each treatment, $n = 32$. For neutralization, Dey-Engley broth was added to the bags containing tissue sections 10 min after treatment. HOBr, hypobromous acid (300 ppm); nASC, neutral acidified sodium chlorite (1,000 ppm); CAB-1, Citrilow (2%); CAB-2, FreshFx (2%).

[c] For each inoculation study, within a serogroup, percentages of recovery from treatments with no common letter are significantly different ($P \leq 0.05$).

recovery rates of the STEC serogroups from tissue samples treated with neutral acidified sodium chlorite ranged from 25 to 50%, which corresponded to the reduction results from the chilled tissue samples in Table 1. Among the treatment groups, the recovery rates of each serogroup of STEC corresponded to the enumerable chilled tissue samples, with some exceptions. For example, the recovery rate of serogroup O26 was significantly lower when treated with neutral acidified sodium chlorite, but not when treated with hypobromous acid and CAB-2, as indicated by the reductions on the chilled tissue samples (Table 1). Similar results were found for the serogroup O145; the recovery rate was lower ($P < 0.05$) for tissue samples treated with neutral acidified sodium chlorite but was the same for the chilled samples treated with CAB-1 and CAB-2. Thus, the recovery of these low inoculation levels of pathogens indicates that there is no single intervention that effectively eliminates them.

In summary, intervention systems are implemented to reduce the likelihood of pathogen occurrence on carcasses and meat products. *E. coli* O157:H7, *Salmonella,* and the top six non-O157 STEC strains are currently the main targets of programs to reduce pathogens on carcasses. Several antimicrobial compounds have been used successfully to reduce pathogens on carcasses in the meat plant, but there are many new commercially available antimicrobial compounds that may be effective and suitable for use in beef plant pathogen-reduction programs. Under the conditions of the study, the general findings indicate that hypobromous acid, neutral acidified sodium chlorite, CAB-1, and CAB-2, when used at concentrations recommended by the manufacturer, could be used to reduce the populations of the pathogens tested on surfaces of fresh beef and could be integrated into the beef industry pathogen-reduction programs. However, low-inoculation results indicated that none of the antimicrobial compounds used in this study eliminated all target pathogenic bacteria; these results are consistent with historical intervention data that indicate the need for a multiple-hurdle approach. The studies were carried out under controlled conditions, rather than in a commercial situation; therefore, the effectiveness of the antimicrobial compounds will need to be validated in actual in-plant conditions.

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