2016

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Journal of Food Protection, Vol. 79, No. 4, 2016, Pages 561–568
doi:10.4315/0362-028X.JFP-15-204

Effect of Exposure Time and Organic Matter on Efficacy of Antimicrobial Compounds against Shiga Toxin–Producing *Escherichia coli* and *Salmonella*

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MS 15-204: Received 8 May 2015/Accepted 6 January 2016

ABSTRACT

Several antimicrobial compounds are in commercial meat processing plants for pathogen control on beef carcasses. However, the efficacy of the method used is influenced by a number of factors, such as spray pressure, temperature, type of chemical and concentration, exposure time, method of application, equipment design, and the stage in the process that the method is applied. The objective of this study was to evaluate effectiveness of time of exposure of various antimicrobial compounds against nine strains of Shiga toxin–producing *Escherichia coli* (STEC) and four strains of *Salmonella* in aqueous antimicrobial solutions with and without organic matter. Non-O157 STEC, STEC O157:H7, and *Salmonella* were exposed to the following aqueous antimicrobial solutions with or without beef purge for 15, 30, 60, 120, 300, 600, and 1,800 s: (i) 2.5% lactic acid, (ii) 4.0% lactic acid, (iii) 2.5% Beefxide, (iv) 1% Aftec 3000, (v) 200 ppm of peracetic acid, (vi) 300 ppm of hypobromous acid, and (vii) water as a control. In general, increasing exposure time to antimicrobial compounds significantly ($P \leq 0.05$) increased the effectiveness against pathogens tested. In aqueous antimicrobial solutions without organic matter, both peracetic acid and hypobromous acid were the most effective in inactivating populations of STEC and *Salmonella,* providing at least 5.0-log reductions with exposure for 15 s. However, in antimicrobials containing organic matter, 4.0% lactic acid was the most effective compound in reducing levels of STEC and *Salmonella,* providing 2- to 3-log reductions with exposure for 15 s. The results of this study indicated that organic matter and exposure time influenced the efficacy of antimicrobial compounds against pathogens, especially with oxidizer compounds. These factors should be considered when choosing an antimicrobial compound for an intervention.

Key words: Antimicrobial compounds; Non-O157 Shiga toxin–producing *Escherichia coli*; *Salmonella*; Shiga toxin–producing *Escherichia coli* O157:H7

Cattle are a major reservoir for *Escherichia coli* O157:H7, which is carried in the intestinal tract of healthy animals and excreted in feces *(8)*. Other organisms of concern to meat processors throughout the red meat supply chain (particularly during packaging and retail) include spoilage microorganisms and pathogens, such as *Salmonella enterica, Listeria monocytogenes,* and *Clostridium perfringens*. All these may be found in the feces and on the hides of cattle presented for slaughter *(16, 17, 34)* and can be transferred to the carcass during harvest, particularly through hide removal (*1, 4, 29*). On 20 October 1999, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) published a final rulemaking in the Federal Register that establishes regulatory sanitation performance standards applicable to all official meat and poultry establishments, including building facilities, processing equipment, workers and practices, pest control, potable water, and sewage and waste disposal control *(38)* as a good manufacturing practice in manufacturing, packing, or holding human food products. However, each processing environment is unique, and in some cases, the methods presented in this document may be inadequate to ensure sanitary conditions or prevent the adulteration of meat and poultry products. The USDA-FSIS has recognized that a decontamination step should be a part of the slaughtering-dressing process *(37)*. To comply with regulatory criteria established by the USDA-FSIS *(37),* the beef industry focuses primarily on meat decontamination through application of various interventions *(2, 27, 36)*. The reason for implementing an intervention is to reduce the pathogenic microorganisms on carcasses and meat with *Salmonella* and *E. coli* O157:H7 as the main target organisms to below the detection limit. There will always be emphasis on continued improvements during the slaughter process, but an alternative long-term strategy may be to minimize the presence of human pathogens on the incoming live animals. Although several preharvest interventions have been developed and evaluated *(6),* for a variety of reasons, widespread implementation of preharvest interventions has not occurred.

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Therefore, postharvest antimicrobial decontamination has been the crucial step to enhance the safety and shelf life of fresh meat. The addition of six more Shiga toxin–producing *Escherichia coli* (STEC) serogroups as adulterants in beef and increased emphasis on *Salmonella* control has resulted in additional research to evaluate antimicrobial interventions for them *(20, 23, 24)*. In applying a microbial reduction step to a carcass, the efficacy of the method used is influenced by factors, such as water pressure, temperature, chemicals present and their concentration, time of exposure, method of application, equipment design, and the stage in the process at which the method is applied (e.g., before hide removal, after hide removal, after evisceration, after chilling) *(2, 27)*. A thorough evaluation of the effects of exposure time and the impact of the high organic load that antimicrobials encounter on the carcass surface would be useful to the fresh beef processing industry. This study evaluated lactic acid (LA; organic acid), Beefxide (BX; combination of organic acids and buffering agent), Aftec 3000 (AF; inorganic acid and its salt), peracetic acid (PAA; acidic oxidizer), and hypobromous acid (BR; neutral oxidizer) that are commonly used in fresh beef processing *(7, 20, 21–24, 33, 35)*. The objective of this study was to evaluate the effectiveness of these antimicrobial compounds against seven serotypes of STEC strains and *Salmonella* in aqueous antimicrobial solutions with and without organic matter with different exposure times. The findings will help the industry identify antimicrobial compounds suitable for interventions at various stages, as well as their effective use (optimized parameters of application in the process) that can significantly improve the safety of meat products.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation of inoculum. Strains of non-O157 *E. coli* serotypes O26:H11/3392, O45:H2/01E-1269, O45/WDG3, O103:H2/2421, O111:NM/1665, O121:H19/O1E-2074, and O145:NM/GS5578620, *E. coli* O157:H7 (ATCC 43895 and FSIS 4), *Salmonella* Newport (13109 and 15124), and *Salmonella* Typhimurium (14218 and DT-104) from the U.S. Meat Animal Research Center 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 5.0×10^8 CFU/ml by using a spectrophotometer at 600 nm *(28)*. A cocktail mixture of an equal volume of each strain of non-O157 STEC, STEC O157:H7, and *Salmonella* was made to form a 13-strain inoculum. The inoculum was diluted with nutrient broth to approximately 1.5×10^7 to 1.5×10^8 CFU/ml.

Aqueous antimicrobial treatments. The antimicrobial compounds that were used in this project are approved as generally recognized as safe, and the concentrations tested were within the recommended range. The following six antimicrobial treatments were prepared at room temperature: (i) 2.5% LA (LA2; pH = 2.4; Purac FCC 88, Corbion, Lenexa, KS), (ii) 4% LA (LA4; pH = 2.2; Corbion), (iii) BX (2.5%; pH = 2.6; Birko Corp., Henderson, CO), (iv) AF (1%; pH = 1.8; Advanced Food Technologies, LLC, Shreveport, LA), (v) PAA (200 ppm, pH = 2.9; Blitz, FMC Corp., Philadelphia, PA), (vi) BR (300 ppm, pH = 6.5; HB2, Enviro Tech Chemical Services, Inc., Modesto, CA), and (vii) sterile deionized water to serve as control. Concentrations of PAA and BR were determined by the manufacturer's recommendation by using a pocket colorimeter II (Hach, Loveland, CO). The antimicrobial treatments were performed by adding 200 μl of inoculum into 800 μl of each antimicrobial compound in a sterile 2-ml biotube (Simport, Beloeil, Canada), mixing with a multichannel pipettor, and exposing for 15, 30, 60, 120, 300, 600, and 1,800 s.

Antimicrobial treatments containing beef purge. Beef purge contains protein and fat and was used to simulate the organic load on the carcass-meat surface. Beef purge (pH = 5.6 to 5.7) 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 counts and *Enterobacteriaceae* counts) of beef purge was approximately 2 to 3×10^3 CFU/ml. In this study, beef purge was prepared to 30% concentration by mixing 30 ml of purge with 70 ml of sterile saline solution. The diluted beef purge was used to prevent protein coagulation when purge was mixed with the antimicrobial compounds. All antimicrobial compounds were prepared as mentioned in the aqueous systems, except preparation with two times concentration (5% LA, 8% LA, 5% BX, 2% AF, 400 ppm of PAA, and 600 ppm of BR) to account for dilution from added purge. The inoculum was prepared by adding 200 μl of two times concentration of bacterial cells (3.0×10^7 to 3.0×10^8 CFU/ml into 800 μl of 30% beef purge. The antimicrobial treatments were performed by mixing 400 μl of beef purge containing inoculum and 400 μl of each antimicrobial compound in a sterile 2-ml biotube (Simport, Beloeil, Canada) and were exposed for 15, 30, 60, 120, 300, 600, and 1,800 s. The average pHs of beef purge containing LA2, LA4, BX, AF, PAA, and BR were 3.0, 2.8, 3.2, 2.7, 4.5, and 6.0, respectively. These preparations yielded the same final concentrations of antimicrobials and inoculum as the treatments without purge.

Microbiological analyses. At the end of each exposure time, 100 μl of treated cell solution was neutralized as follows: with 900 μl of Dey-Engley broth (BD) for antimicrobial treatment alone and with 900 μl of two times concentration of Dey-Engley for antimicrobial treatments with purge. The Dey-Engley broth either with or without purge was held at room temperature for 1 h to allow subleathal injured cells to recover *(30)* and after resuscitation, 10-fold serially diluted with maximum recovery diluents (BD). Appropriate dilutions were surface plated on the U.S. Meat Animal Research Center chromogenic agar medium *(25)* by using a spiral plater (Spiral Biotech, Advanced Instruments, Inc., Norwood, MA). All plates were incubated at 37°C for 24 h and at room temperature for 30 min for full color development for enumeration. Colony colors developed on the U.S. Meat Animal Research Center chromogenic agar medium were turquoise blue, blue-green, light green, dark blue green, light blue gray, purple, hunter green, and colorless with a magenta halo around the colony for O26, O45, O103, O111, O121, O145, O157, and *Salmonella*, respectively. The limit of detection using a spiral plater was 10 CFU/ml. CFU from untreated control (water) and treated samples were counted from the U.S. Meat Animal Research Center chromogenic agar plates. Two colored colonies representing each STEC serogroup and *Salmonella* were picked for confirmation. STECs were confirmed by using multiplex PCR *(13, 18, 19, 31)*. A separate multiplex PCR *(26)* was used to confirm *Salmonella*.

Statistical analyses. Bacterial populations of untreated control and treated samples were transformed to log CFU per milliliter values from six experimental replications of each treatment. One-way statistical analysis (analysis of variance) was

performed by using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC). Least-squares means were calculated, and pairwise comparisons of exposure time means were determined using the Tukey-Kramer test method with a probability level at $P \leq 0.05$.

RESULTS AND DISCUSSION

Reductions of non-O157 STEC, STEC O157:H7, and *Salmonella* following treatment with antimicrobial compounds at different exposure times. The reductions of non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145, STEC O157, and *Salmonella* with antimicrobial solutions depended on the antimicrobial compound used, as well as exposure time of the treatment (Table 1). Treatment with 2 or 4% LA for 15 s resulted in reductions of non-O157 STEC, STEC O157, and *Salmonella* that ranged from 1.2 to 2.2 log CFU/ml or from 2.4 to 3.3 log CFU/ml, respectively. With treatment of LA2 for 300 s or LA4 for 120 s, approximately 5-log CFU/ml reductions of these pathogens were found, with no increase in inactivation ($P > 0.05$) after these exposure times. Treatment with BX for 120 s resulted in reductions of STEC and *Salmonella,* ranging from 0.9 to 1.9 log CFU/ml, with 2- to 3-log reductions when exposure time was 600 s and 3- to 5-log reductions after 1,800 s. The AF treatment for 30 s reduced the populations of STEC and *Salmonella* by 1.2 to 2.4 log CFU/ml, and both pathogens were reduced ($P < 0.05$) by more than 3 log after increasing exposure time to 120 s and 4 to 6 log, with 300 to 1,800 s exposure time. In contrast, PAA and BR reduced STEC and *Salmonella* at least 5 log CFU/ml, with 15 s of treatment, and showed no further reduction ($P > 0.05$) of these pathogens after increasing exposure time from 30 to 1,800 s.

Reductions of non-O157 STEC, STEC O157:H7, and *Salmonella* following treatment with antimicrobial compounds containing beef purge at different exposure times. The high organic matter from added purge had little effect on reductions of non-O157 serogroups O26, O45, O103, O111, O121, and O145, STEC O157, and *Salmonella* by LA4 ($P > 0.05$; Table 2). Reductions by LA2, BX, and AF followed a similar, but delayed trend, with exposure time for treatments without purge. Treatment with PAA and BR in the presence of beef purge resulted in reductions of non-O157 STEC, STEC O157, and *Salmonella* of more than 1.5 log CFU/ml and 0.1 log CFU/ml, respectively. Efficacy of PAA in beef purge was less than the efficacy of these compounds in aqueous solution at exposure times less than 60 s. BR in purge did not reduce levels of STEC or *Salmonella,* regardless of exposure time. Similar reductions of STEC and *Salmonella* from PAA with or without beef purge was found after exposure for 300 s. Treatment with LA2 resulted in reductions of STEC and *Salmonella* that ranged 1.2 to 2.1 log CFU/ml (except serogroup O45) after exposure for 30 s, which was twice the exposure time to obtain the same reductions in aqueous solution without purge. In contrast, treatment with LA4 in the presence of beef purge resulted in similar reductions of STEC and *Salmonella* as found in the aqueous solution at the same exposure times. Similar to treatment with LA2, the reduction of STEC and *Salmonella* by using BX or AF containing beef purge required 180 or 30 s more exposure time, respectively, compared with the same compounds without purge, except for serogroup O45 (Tables 1 and 2).

Efficacy of antimicrobial compounds against pathogens in antimicrobial compounds with and without purge. To determine which antimicrobial compounds more effectively reduced non-O157 STEC, STEC O157, or *Salmonella,* the data were pooled together, regardless of treatment times before analyses. Effectiveness of LA, BX, AF, PAA, and BR against these pathogens was reduced in the presence of high organic load compared with aqueous antimicrobial solutions, especially for PAA and BR (Fig. 1A and 1B). In aqueous solutions, PAA and BR inactivated non-O157 STEC, STEC O157, and *Salmonella* by approximately 5.6 log CFU/ml, while the same compounds in beef purge inactivated these pathogens by approximately 3.5 and 0.1 log CFU/ml, respectively. Pathogen reductions using AF with purge were slightly lower than without purge (3.2 versus 3.7 log CFU/ml). However, the inactivation of LA (2 and 4%) and BX for these pathogens in aqueous solutions was approximately 4.0, 5.0, and 2.0 log CFU/ml, respectively, and similar to reductions of these antimicrobial compounds with purge (Fig. 1A and 1B). In aqueous antimicrobial solutions (Fig. 1A), the average reductions of non-O157 STEC, STEC O157, and *Salmonella* due to PAA and BR were similar ($P > 0.05$) but greater ($P < 0.05$) than LA4. The average reductions of these pathogens due to LA2 and AF also were equally effective but less than PAA, BR, and LA4, while BX was the least effective in reducing these pathogens (Fig. 1A). In contrast, with high organic load, LA4 was the most effective ($P < 0.05$), and BR was the least effective in reducing non-O157 STEC, STEC O157, and *Salmonella* (Fig. 1B). The average reductions of these pathogens due to PAA, LA2, and AF with purge were similar ($P > 0.05$) and higher than BX with purge but less than the reduction of LA4 with purge.

Numerous antimicrobial compounds have been used to enhance the safety and shelf life of food. The amounts and types of chemical compounds that could be used for human consumption are governed by regulatory agencies and include not only the amounts and types of chemical compounds used but also the methods for delivery, organic matter encountered, and exposure time of these compounds *(12)* may ultimately affect their effectiveness. In this study, beef purge was included to represent the high organic matter encountered by antimicrobials used to treat fresh meat products. In general, a longer exposure time and a higher concentration of antimicrobial solution increased the reduction of non-O157 STEC, STEC O157, and *Salmonella*. LA is one of the most widely studied and used organic acids for antimicrobial interventions in the U.S. beef industry *(7, 9, 12, 23, 32, 33).* Recently, LA has been approved for use as an antimicrobial compound during processing beef cattle in European countries *(39).* The effect of the use of LA varies among studies but generally suggests the achievement of a 1.0- to 2.0-log reduction. In this study, both concentrations of LA reduced populations of STEC and *Salmonella* by more than 1 log CFU/ml after 15 s with greater reductions up to 120 to

TABLE 1. *Reduction of STEC and* Salmonella *strains following treatment with antimicrobial aqueous solution*[a]

Compound[b]	Strain	Mean reduction (log CFU/ml) following treatment for indicated duration						
		15 s	30 s	60 s	120 s	300 s	600 s	1,800 s
LA2	O26	2.2 D[c]	3.0 CD	3.7 BC	4.6 AB	5.7 A	5.7 A	5.7 A
	O45	1.2 C	1.8 BC	2.3 BC	2.8 B	4.6 A	5.2 A	5.3 A
	O103	2.0 C	2.5 C	3.4 B	3.6 B	5.9 A	5.9 A	5.9 A
	O111	1.2 D	1.7 CD	2.7 BC	3.7 B	5.9 A	5.9 A	5.9 A
	O121	1.5 C	1.7 BC	2.3 BC	3.2 B	4.6 AB	5.5 A	5.5 A
	O145	1.8 D	2.4 D	3.4 C	4.6 B	5.9 A	5.9 A	5.9 A
	O157	1.3 D	1.7 CD	2.2 CD	2.6 C	4.0 B	5.1 A	5.7 A
	Salmonella	1.5 D	1.9 D	2.8 CD	3.9 BC	5.0 AB	5.6 A	5.6 A
LA4	O26	3.3 C	3.8 BC	4.8 AB	5.4 A	5.6 A	5.6 A	5.6 A
	O45	2.4 D	2.8 CD	3.9 BC	4.8 AB	5.2 A	5.2 A	5.2 A
	O103	2.8 C	3.6 BC	4.7 AB	5.6 A	5.6 A	5.6 A	5.6 A
	O111	2.7 B	3.5 B	5.2 A	5.8 A	5.8 A	5.8 A	5.8 A
	O121	2.5 B	2.9 B	4.0 AB	5.1 A	5.4 A	5.4 A	5.4 A
	O145	3.3 C	4.0 BC	5.0 AB	5.8 A	5.8 A	5.8 A	5.8 A
	O157	2.4 B	2.6 B	3.2 B	5.0 A	5.8 A	5.8 A	5.8 A
	Salmonella	2.6 B	3.4 B	5.0 A	5.7 A	5.7 A	5.7 A	5.7 A
BX	O26	0.5 D	0.9 CD	1.0 CD	1.3 CD	2.0 C	3.7 B	5.4 A
	O45	0.3 D	0.5 CD	0.6 CD	0.9 CD	1.7 BC	2.7 B	4.0 A
	O103	0.7 D	1.0 D	1.1 CD	1.4 CD	2.0 C	3.2 B	4.2 A
	O111	0.3 C	0.4 C	0.6 C	0.9 BC	1.8 B	3.2 A	4.0 A
	O121	0.6 B	0.9 B	0.7 B	1.2 B	1.7 AB	2.5 A	3.4 A
	O145	0.1 D	0.4 D	0.7 D	1.0 CD	2.2 BC	3.4 B	5.4 A
	O157	0.3 D	0.7 D	0.9 CD	1.2 CD	1.7 BC	2.2 B	3.9 A
	Salmonella	0.4 D	0.7 D	1.3 CD	1.9 CD	2.7 BC	3.5 B	5.3 A
AF	O26	0.9 C	1.9 C	3.3 B	4.4 B	5.8 A	5.8 A	5.8 A
	O45	0.6 D	1.3 D	2.6 C	3.9 B	5.0 AB	5.3 A	5.3 A
	O103	0.8 D	1.4 CD	2.6 C	4.3 B	5.6 A	5.7 A	5.7 A
	O111	0.6 E	1.2 DE	2.1 D	3.2 C	4.7 B	5.2 AB	6.0 A
	O121	0.6 D	1.5 CD	2.2 C	3.7 B	4.9 AB	5.1 A	5.3 A
	O145	1.1 E	2.2 D	3.6 C	4.6 BC	5.4 AB	6.0 A	6.0 A
	O157	0.7 E	1.7 E	2.3 D	3.2 C	4.4 B	5.4 A	5.8 A
	Salmonella	1.1 D	2.4 C	3.3 B	5.0 A	5.8 A	5.8 A	5.8 A
PAA	O26	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A
	O45	5.4 A	5.4 A	5.4 A	5.4 A	5.4 A	5.4 A	5.4 A
	O103	5.3 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A
	O111	5.7 A	6.0 A	6.0 A	6.0 A	6.0 A	6.0 A	6.0 A
	O121	5.5 A	5.5 A	5.5 A	5.5 A	5.5 A	5.5 A	5.5 A
	O145	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A
	O157	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A
	Salmonella	5.8 A	5.8 A	5.8 A	5.8 A	5.8 A	5.8 A	5.8 A
BR	O26	5.6 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A
	O45	5.0 A	5.3 A	5.3 A	5.3 A	5.3 A	5.3 A	5.3 A
	O103	5.3 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A	5.6 A
	O111	5.6 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A
	O121	5.0 A	5.3 A	5.3 A	5.3 A	5.3 A	5.3 A	5.3 A
	O145	5.7 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A	5.9 A
	O157	5.2 A	5.7 A	5.7 A	5.7 A	5.7 A	5.7 A	5.7 A
	Salmonella	5.2 A	5.5 A	5.5 A	5.5 A	5.5 A	5.5 A	5.5 A

[a] Non-O157 serogroups O26, O45, O103, O111, O121, and O145; O157, *E. coli* O157:H7.

[b] LA, lactic acid, 2 or 4%; BX, Beefxide 2% (wt/vol); AF, Aftec 3000 2% (vol/vol); PAA, 200 ppm of peroxyacetic acid; BR, 300 ppm of hypobromous acid.

[c] Means in the same row within each antimicrobial compound and bacterial strain bearing no common letter are significantly different ($P < 0.05$). For each value, $n = 6$.

300 s exposure time (Tables 1 and 2). Schmidt et al. *(35)* reported that LA had greater reductions of STEC and *Salmonella* on inoculated cheek meat, with increasing exposure time from 1 to 5 min. Similarly, a greater reduction of STEC O157:H7 on beef heads treated with LA2 was found with an exposure time of 26 s compared with 12 s *(21)*. With the high organic load of beef purge, less reduction of only non-O157 STEC was observed with LA2 at lower exposure

TABLE 2. *Reduction of STEC and* Salmonella *strains following treatment with purge containing antimicrobial*[a]

Compound	Strain	Mean reduction (log CFU/ml) following treatment for indicated duration						
		15 s	30 s	60 s	120 s	300 s	600 s	1,800 s
LA2[b]	O26	1.5 D[c]	2.1 CD	3.2 BC	4.0 B	5.2 AB	5.8 A	5.8 A
	O45	0.6 E	0.8 E	1.2 DE	2.5 CD	3.4 BC	4.0 B	5.8 A
	O103	1.3 E	1.7 DE	2.7 CD	3.4 C	4.5 B	5.6 A	5.6 A
	O111	0.9 C	1.6 C	2.8 B	3.8 B	5.4 A	5.8 A	5.8 A
	O121	0.7 E	1.2 DE	1.6 DE	2.2 CD	3.2 BC	3.7 B	5.7 A
	O145	1.0 D	1.6 D	2.7 C	3.1 C	4.6 B	5.8 A	5.8 A
	O157	1.0 D	1.9 CD	2.6 C	4.3 B	5.6 A	6.0 A	6.0 A
	Salmonella	1.2 C	2.0 BC	2.9 B	4.6 A	5.4 A	5.9 A	5.9 A
LA4	O26	3.2 B	3.5 B	5.3 A	5.3 A	5.8 A	5.8 A	5.8 A
	O45	1.8 C	2.3 BC	3.4 B	4.6 A	5.3 A	5.5 A	5.5 A
	O103	2.9 B	3.2 B	5.1 A	5.1 A	5.6 A	5.6 A	5.6 A
	O111	3.1 B	3.5 B	5.2 A	5.4 A	5.8 A	5.8 A	5.8 A
	O121	2.0 C	2.7 BC	3.2 B	5.0 A	5.8 A	5.8 A	5.8 A
	O145	3.0 B	3.6 B	5.4 A	5.6 A	5.8 A	5.8 A	5.8 A
	O157	2.8 B	3.0 B	3.3 B	5.6 A	6.0 A	6.0 A	6.0 A
	Salmonella	2.8 B	3.0 B	3.6 B	5.8 A	6.0 A	6.0 A	6.0 A
BX	O26	0.2 D	0.8 D	0.5 D	0.8 D	2.0 C	2.9 B	5.2 A
	O45	0.1 D	0.3 D	0.2 D	0.7 CD	0.8 C	1.3 B	2.8 A
	O103	0.5 D	0.9 CD	0.8 CD	1.3 CD	1.8 C	3.2 B	5.0 A
	O111	0.3 D	0.6 D	0.5 D	0.9 D	2.2 C	3.1 B	5.4 A
	O121	0.2 D	0.5 D	0.3 D	0.6 CD	1.0 C	1.7 B	3.4 A
	O145	0.2 D	0.5 CD	0.3 CD	0.5 CD	1.5 C	3.2 B	5.1 A
	O157	0.3 C	0.9 C	0.5 C	0.8 C	1.9 B	2.9 B	4.9 A
	Salmonella	0.4 E	1.5 D	1.2 D	1.8 CD	2.3 C	3.3 B	5.7 A
AF	O26	0.3 D	0.9 CD	1.9 BC	3.1 B	4.7 A	4.8 A	5.4 A
	O45	0.2 D	0.3 D	0.5 CD	1.4 C	2.5 B	2.9 B	4.1 A
	O103	0.4 F	0.8 EF	1.7 DE	2.4 CD	3.4 BC	4.5 AB	4.8 A
	O111	0.2 D	0.6 D	1.9 C	3.2 B	4.8 A	5.1 A	5.7 A
	O121	0.2 D	0.5 D	1.3 C	2.0 C	3.2 B	3.5 AB	4.3 A
	O145	0.2 E	0.5 E	1.6 D	2.4 CD	3.5 B	3.9 B	4.9 A
	O157	0.5 E	0.9 E	2.0 D	3.1 C	4.5 B	5.6 A	6.0 A
	Salmonella	1.1 D	1.5 D	2.4 C	3.4 B	5.8 A	6.0 A	6.0 A
PAA	O26	2.0 B	2.8 B	5.1 A	5.6 A	5.8 A	5.8 A	5.8 A
	O45	1.6 B	2.1 B	3.0 A	3.6 A	4.0 A	4.1 A	4.2 A
	O103	2.0 B	2.5 B	4.4 A	4.6 A	4.9 A	5.2 A	5.4 A
	O111	2.3 B	3.2 B	5.0 A	5.7 A	5.9 A	5.9 A	5.9 A
	O121	1.9 B	2.6 B	4.6 A	4.8 A	5.8 A	5.8 A	5.8 A
	O145	1.7 B	2.5 B	4.6 A	5.4 A	5.9 A	5.9 A	5.9 A
	O157	1.6 C	2.4 BC	2.9 B	3.2 AB	3.5 A	3.8 A	4.0 A
	Salmonella	1.7 B	2.2 B	3.2 A	3.3 A	3.6 A	3.9 A	4.3 A
BR	O26	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.2 A
	O45	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.2 A
	O103	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.2 A	0.2 A
	O111	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.1 A	0.1 A
	O121	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A
	O145	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A	0.1 A
	O157	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.1 A	0.2 A
	Salmonella	0.1 A	0.1 A	0.1 A	0.1 A	0.2 A	0.2 A	0.2 A

[a] Non-O157 serogroups O26, O45, O103, O111, O121, and O145; O157, *E. coli* O157:H7.

[b] LA, lactic acid 2 or 4%; BX, Beefxide 2% (wt/vol); AF, Aftec 3000 2% (vol/vol); PAA, 200 ppm of peroxyacetic acid; BR, 300 ppm of hypobromous acid.

[c] Means in the same row within each antimicrobial compound and bacterial strain bearing no common letter are significantly different ($P < 0.05$). For each value, $n = 6$.

times (Table 2). However, beef purge had no effect on reductions of non-O157 STEC, STEC O157, and *Salmonella* when exposed to LA4, as indicated by a similar reduction of pathogens from each exposure time.

BX and AF were less effective in reducing non-O157 STEC, STEC O157, and *Salmonella* both in aqueous and in beef purge containing antimicrobial compounds compared with LA4. To reduce at least 1 log CFU/ml of STEC

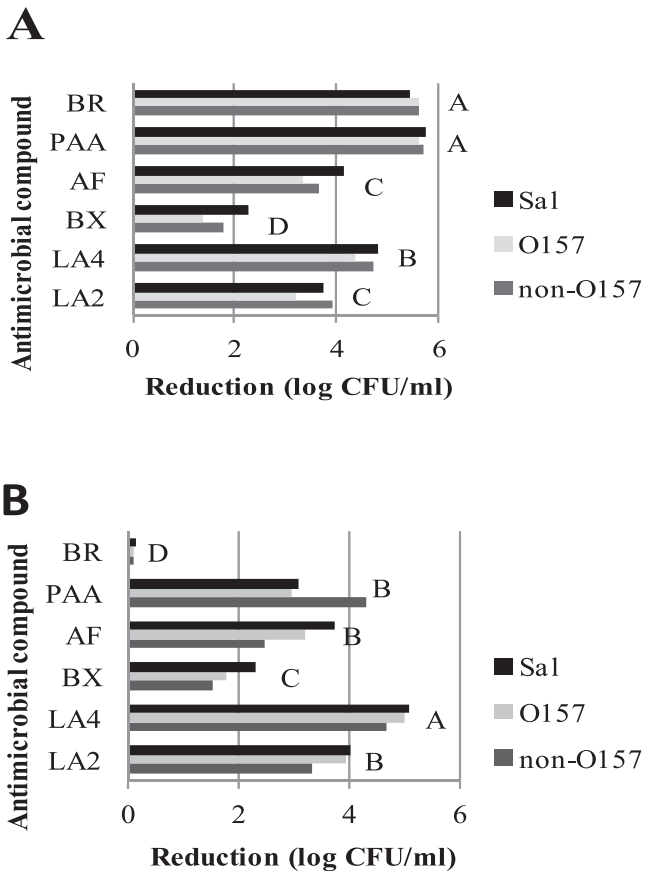

FIGURE 1. *Effectiveness of each antimicrobial compound*[a] *against pathogenic bacteria*[b] *in aqueous solution (A) and beef purge system (B).*[c] [a] *LA, lactic acid 2 or 4%; BX, Beefxide 2% (wt/vol); AF, Aftec 3000 2% (vol/vol); PAA, 200 ppm of peroxyacetic acid; BR, 300 ppm of hypobromous acid.* [b] *The log reduction data for each bacterial strain were pooled together with all exposure times and averaged the reduction data for all pathogens in each treatment group for statistical analysis. Sal,* Salmonella*; O157, STEC O157:H7; non-O157, non-O157 STEC.* [c] *Means between each antimicrobial compound bearing no common letter (A through D) are significantly different (*$P < 0.05$*).*

and *Salmonella,* the exposure times for BX and AF were 120 and 30 s in aqueous solutions and 300 and 60 s in these two compounds containing beef purge, respectively (Tables 1 and 2). Similar results were reported when cheek meat inoculated with STEC and *Salmonella* were treated with BX and AF *(35)*. LA, BX, and AF have been used as antimicrobial interventions to inactivate pathogens associated with meat during processing. LA was more effective because LA is lipophilic and enters freely through the cell membrane as a function of concentration gradient *(11)*. Inside the cells, LA dissociates causing interference with nutrient transport and energy generation and causes low internal pH that damages cellular macromolecules, and subsequently, kills the bacterial cells *(15)*. BX is composed of lactic and citric acids and potassium hydroxide and is less effective than LA alone due to citric acid and the buffering effect of potassium hydroxide. Citric acid reduces the amount of available LA, and the antibacterial effect of citric acid is from a different mechanism than from a lipophilic acid such as LA. The antibacterial effect of citric acid is partially due to its ability to chelate divalent cations, and meat has sufficient divalent cations to neutralize this effect *(3, 14)*. AF is composed of sulfuric acid (inorganic acid) and sodium sulfate. The rapid dissociation rate of sulfuric acid causes environmental pH to drop as a result of increasing proton concentration [H^+], which interferes with the transmembrane proton gradient of the microbial cells *(3, 5)*. Bacterial cells overcome the increasing proton concentration of environmental pH by transportation of protons through the proton pump into the cells for neutralization. The transportation of protons using a proton pump causes (a) depletion in the cells' energy and (b) a decrease in the internal pH of the cells due to transported protons *(15)*. Exposure to [H^+] adversely affects the ionic bonds of the macromolecules and can interfere with their three-dimensional structures *(5, 15)*.

Both PAA and BR are classified as oxidizers. The effectiveness of PAA and BR, in general, against non-O157 STEC, STEC O157:H7, and *Salmonella* depended on the environment surrounding the pathogens. In aqueous solution, both PAA and BR reduced all pathogens tested by more than 5 log CFU/ml within 15 s, while the same antimicrobial compounds with high organic load of beef purge and 15 s exposure time were far less effective. In fact, BR was not effective at any exposure time, while PAA had reduced effectiveness at the shorter exposure times. The primary antimicrobial action of oxidizers against bacterial cells is due to the oxidizing effect on the thiol group (–SH) in many enzymes and structural proteins and interference with metabolism *(10)*. However, the oxidizing of protein and fat from beef purge reduces the availability of the oxidizer and could be a reason why PAA and BR were less effective in the presence of high organic matter. Other studies *(20, 25, 35)* with fresh beef inoculated with the aforementioned pathogens and immersed in different antimicrobial compounds found that PAA and BR reduced populations of pathogens by a 0.3- to 0.7-log reduction within 30 to 60 s. In contrast, when inoculated fresh beef was treated with PAA and BR by spraying instead of immersion, more than 1-log reduction of non-O157 STEC, STEC O157:H7, and *Salmonella* has been reported *(23, 25)*. This may mean that further investigation of the methods of application for these two compounds is needed to overcome effects of high organic load when treating meat surfaces.

Overall, the reduction data for antimicrobial compounds with and without beef purge were pooled across exposure times, and average reduction data for each pathogen and antimicrobial combination were analyzed to compare inactivation efficiency in different environments (Fig. 1A and 1B). Without beef purge, the order of efficacy was PAA = BR > LA4 > LA2 = AF > BX, whereas the order of efficacy was LA4 > LA2 = PAA = AF > BX > BR in beef purge containing antimicrobial compounds.

In conclusion, the results of this study indicated that the reductions of non-O157 STEC, STEC O157:H7, and *Salmonella* under the conditions examined depended on

exposure time, the type of antimicrobial compound used, bacterial strains, and organic load. Organic matter strongly influenced the effectiveness of some antimicrobial compounds, especially with oxidizers or compounds containing oxidizers. These data indicate that the application of antimicrobial compounds on meat surfaces under commercial conditions likely will result in greater reductions with short exposure time for LA4. However, after 60 s, LA4 and PAA will have similar effects that will be greater than for other compounds tested here. Application of BX or AF may be more suitable for a longer contact time, such as subprimal cuts, before packing or for trimmings than for carcasses that have a shorter exposure time due to subsequent washing steps. The studies were carried out under controlled conditions, rather than in a commercial situation; therefore, the validation of the antimicrobial compounds under actual in-plant conditions will, ultimately, be necessary.

ACKNOWLEDGMENTS

The authors thank Corbion, Birko Corp., Enviro Tech Chemical Services, FMC Corp., and Advanced Food Technologies, LLC, for providing their products and technical support. The authors also thank Bruce Jasch for his laboratory technical support and Jody Gallagher for her secretarial support. 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.

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