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Research Paper

Efficacy of Antimicrobial Interventions Used in Meat Processing Plants against Antimicrobial Tolerant Non–Antibiotic-Resistant and Antibiotic-Resistant *Salmonella* on Fresh Beef

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ABSTRACT

Salmonella is a common cause of foodborne illness in the United States, and several strains of *Salmonella* have been identified as resistant to antibiotics. It is not known whether strains that are antibiotic resistant (ABR) and that have some tolerance to antimicrobial compounds are also able to resist the inactivation effects of antimicrobial interventions used in fresh meat processing. Sixty-eight *Salmonella* isolates (non-ABR and ABR strains) were treated with half concentrations of lactic acid (LA), peracetic acid (PAA), and cetylpyridinium chloride (CPC), which are used in beef processing plants to screen for tolerant strains. Six strains each from non-ABR and ABR *Salmonella* that were most tolerant of LA (2%), PAA (200 ppm), and CPC (0.4%) were selected. Selected strains were inoculated on surfaces of fresh beef and subjected to spray wash treatment with 4% LA, 400 ppm PAA, or 0.8% CPC for the challenge study. Tissue samples were collected before and after each antimicrobial treatment for enumeration of survivors. Spray treatment with LA, PAA, or CPC significantly reduced non-ABR *Salmonella* and ABR *Salmonella* on surfaces of fresh beef by 1.95, 1.22, and 1.33 log CFU/cm^2, and 2.14, 1.45, and 1.43 log CFU/cm^2, respectively. The order of effectiveness was LA > PAA = CPC. The findings also indicated that LA, PAA, and CPC were equally ($P \leq 0.05$) effective against non-ABR and ABR *Salmonella* on surfaces of fresh beef. These data contribute to the body of work that indicates that foodborne pathogens that have acquired both antibiotic resistance and antimicrobial tolerance are still equally susceptible to meat processing antimicrobial intervention treatments.

HIGHLIGHTS

- LA, PAA, or CPC equally reduced antimicrobial tolerant non-ABR and ABR *Salmonella*.
- LA was the most effective in reducing *Salmonella* on fresh beef surfaces.
- CPC has the potential to be used as a beef carcasses decontamination agent.

Key words: Antimicrobial tolerance; Cetylpyridinium chloride; Fresh beef; Lactic acid; Non-antibiotic resistant and antibiotic resistant *Salmonella*; Peracetic acid

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Salmonella is a common cause of foodborne illness in the United States. Based on the White–Kauffman–Le Minor scheme, more than 2,600 *Salmonella* serovars have been identified; most of the serotypes belong to *S. enterica (37)*. *S. enterica* is composed of six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae,* and *indica*. The subspecies *enterica* is responsible for approximately 1,547 serovars that can cause infections in warm-blooded animals and humans *(21)*. A meta-analysis of the diversity of *S. enterica* serovars worldwide indicated that serovars Typhimurium, Enteritidis, Anatum, Derby, Sofia, Hadar, Agona, Weltevreden, Meleagridis, Infantis, and Kentucky were the most frequent causes of human disease associated with beef, pork, chicken, and seafood *(16)*. The Centers for Disease Control and Prevention estimates one million *Salmonella* illnesses occur annually in the United States, resulting in 26,500 hospitalizations and 420 deaths *(10)*. Of all salmonellosis cases, approximately one-third are attributable to food produced under inspection by the Food Safety and Inspection Service of the U.S. Department of Agriculture. Beef products account for approximately 10% of foodborne *Salmonella* cases. In 2016, the most common serotypes from clinical isolates associated with cattle were *Salmonella* serovars Dublin, Cerro, Typhimurium, Montevideo, and Heidelburg *(32)*.

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Cattle and swine are reservoirs of *Salmonella* serovars that can contaminate meat products. Feedlot cattle presented for slaughter were sampled from spring 2001 to winter 2002; the *Salmonella* fecal prevalence ranged from 2.1 to 9.1% *(3)*. In 2002, U.S. dairy cattle were shown to have a *Salmonella* fecal prevalence of 7.3% *(40)*. As with other pathogens shed in feces, *Salmonella* pathogens have been found at higher rates on the hides of cattle than in feces. Hide prevalence has been reported to reach levels of 91.6 and 97.7% in the summer and fall, respectively *(3)*. In a study from July 2005 to April 2006, the prevalence of *Salmonella* on preevisceration and postintervention carcasses was 50.2 and 0.8%, respectively *(8)*. The prevalence of *Salmonella* in commercial ground beef from July 2005 to June 2007 was found to be 4.2%; it varied by region and month from 1.8 to 6.5% *(5)*.

The prevalence of human bacterial infections resistant to antibiotic therapy has been recognized as a critical global public health concern by governmental, professional, medical, and scientific organizations *(9, 13, 39, 43, 44)*. It is generally accepted that bacterial antibiotic resistance is a naturally occurring phenomenon. Research to develop ways to reduce the levels of *Salmonella* on farms and in foods is important, as is understanding and reducing the risk posed to food safety by antibiotic-resistant (ABR) bacteria present in the meat production system. Antimicrobial interventions reduce pathogens and spoilage bacteria and, consequently, lead to improved safety and shelf life of fresh or further processed products *(2, 23)*. Lactic acid (LA) and peracetic acid (PAA) are the two antimicrobial compounds currently most used in the meat industry. There is some concern that ABR *Salmonella* might also be more resistant to antimicrobial interventions used in beef processing to reduce the risk of foodborne pathogens *(2)*. In addition, acid resistance and/or acid tolerance of pathogens may enhance survival from interventions and, subsequently, cause illnesses *(4, 19, 34)*. *Salmonella* strains that have shown resistance to both antibiotics and acidic conditions have been isolated from animals, foods, and wastewater *(11)*. Isolates with acid and antibiotic resistance could have significant implications for the meat industry and need to be further investigated. However, it is not fully known whether ABR *Salmonella* strains that are tolerant of antimicrobials are able to resist the bactericidal effects of the antimicrobial interventions used in beef processing. The objectives of this research project were to determine the efficacy of antimicrobial interventions on reduction of *Salmonella* load on beef carcass surfaces and to determine whether the interventions currently used in the processing facilities are sufficient to reduce the *Salmonella* load of beef carcasses contaminated with ABR strains of *Salmonella* that also are tolerant of these antimicrobials.

MATERIALS AND METHODS

***Salmonella* isolates and growth conditions.** The 68 *Salmonella* isolates used in this study were obtained from the U.S. Meat Animal Research Center (USMARC) strains collection, and were composed of 33 ABR isolates and 35 non-ABR isolates (Table 1). Most of the isolates were obtained from samples of feedlot animals, holding pen animals, and beef carcasses at the processing plants. The *Salmonella* isolates were confirmed using gram negative identification plates (Trek Diagnostic Systems, Cleveland, OH) according to the manufacturer's recommendations. The isolates were then subcultured on tryptic soy agar (TSA) plates for MIC determination using National Antibiotic Resistance Monitoring System antibiotic susceptibility panels (Trek Diagnostic Systems). MICs were set up using a Sensititre auto diluter system (Trek Diagnostic Systems) according to the manufacturer's directions. After the antibiotic susceptibility panels were incubated at 37°C for 18 to 24 h, MIC was determined using the Sensititre Autoreader System (Trek Diagnostic Systems). The isolates were serogrouped with latex agglutination kits (Wellcolex Colour Salmonella, Remel, Lenexa, KS). After serogroups were identified, each isolate was serotyped with PCR of common first- and second-phase flagellar antigens *(15, 20)*. Results of flagellar antigen PCR were confirmed with specific antisera (Remel) according to the manufacturer's instructions. Each isolate was grown statically in nutrient broth (Difco, BD, Franklin Lakes, NJ) for 16 to 18 h at 37°C. The cell density of each isolate was determined and adjusted to a cell concentration of approximately 3×10^8 cells per mL using a spectrophotometer at 600 nm *(31)*. The isolates were screened against antimicrobials to determine their resistance or tolerance (lower reduction compared with untreated control of the same isolates) to each compound.

Screening *Salmonella* isolates for resistance and/or tolerance to antimicrobial interventions. An aliquot of 200 μL of each isolate was mixed with 400 μL of fresh beef purge (mostly water containing various nutrients that accumulates inside a package of meat) as the stock *Salmonella* solution before screening with each antimicrobial compound. Beef purge was aseptically collected from multiple vacuum-packaged beef subprimals obtained from a local beef cattle processing plant that had been frozen (–20°C) and then thawed (4°C) three times to increase the amount of purge. The combined beef purge had approximately 2×10^3 cells per mL of aerobic bacteria using aerobic count plate (3M Petrifilm, St. Paul, MN). The aerobic count plates were incubated at 25°C for 2 h for resuscitation of injured cells due to freeze and thaw cycles and were incubated at 35°C for 48 h. The top films from aerobic count plate were placed on xylose lysine deoxycholate (XLD; Oxoid, Thermo Fisher Scientific, Waltham, MA) and incubated at 37°C for 24 h to detect *Salmonella*. The combined beef purge that contained no *Salmonella* was used for the study. Beef purge was used to simulate the organic matter on surfaces of fresh beef *(25)* and was diluted to 40% beef purge with sterile saline solution to prevent soluble proteins coagulation when mixed with acidic antimicrobial compounds. Aliquots of 400 μL of beef purge containing each isolate were placed in 2-mL sterile cluster tubes (BioTube T105, Simport Scientific, Quebec, Canada) and treated by mixing with 400 μL of the following antimicrobial compounds for 0 s (initial population) and 30 s: (i) sterile water as a control, (ii) 4% LA (Purac-88%, Corbion, Lenexa, KS), (iii) 400 ppm of PAA (Blitz, PeroxyChem, Philadelphia, PA), and (iv) 0.8% cetylpyridinium chloride (CPC; Sigma-Aldrich, St. Louis, MO). Using 1:1 (v/v) mixtures of individual beef purge–isolate and antimicrobial compounds, the final concentrations of LA, PAA, and CPC were 2%, 200 ppm, and 0.4%, respectively, which are half strength of the concentrations commonly used by the meat industry. At the meat plant, carcasses are spray treated with antimicrobials at full strength for 10 to 15 s. Lower concentrations (0.5 or 1%) of antimicrobials will result in less inactivation and may lead to increases in false tolerant strains. Therefore, antimicrobials were used at half strength and contact time was increased to 30 s for the screening. The final concentration of beef

TABLE 1. Salmonella *isolates, sources, ABR, and reduction to half concentration of antimicrobials for developing resistant and/or tolerant* Salmonella *strains*[a]

Salmonella	Designation	Source[b]	ABR[c]	Log reduction (CFU/mL)[d]		
				LA	PAA	CPC
Agona	**NS-125**	**USMARC (cattle hide)**	**N**	**0.17 (0.05)**	**0.25 (0.10)**	**0.58 (0.14)**
Agona	**CS-120**	**USMARC (cattle feces)**	**N**	**0.19 (0.05)**	**0.32 (0.24)**	**0.29 (0.13)**
Anatum	APHIS 94-6411	USDA APHIS	N	0.33 (0.30)	0.55 (0.25)	0.84 (0.14)
Anatum	NS-114	USMARC (cattle hide)	N	0.17 (0.02)	1.56 (1.18)	0.55 (0.15)
Anatum	CS-45	USMARC (cattle hide)	N	0.52 (0.57)	3.87 (1.05)	0.85 (0.09)
Anatum	O1-E8	USMARC (lymph node)	N	0.29 (0.13)	0.75 (0.50)	0.39 (0.29)
Cerro	APHIS 94-6513	USDA APHIS	N	0.27 (0.18)	2.33 (2.16)	0.66 (0.28)
Cerro	**O1-A4**	**USMARC (lymph node)**	**N**	**0.25 (0.16)**	**0.51 (0.52)**	**0.50 (0.26)**
Dublin	95-11800	USDA APHIS (NVSL)	N	0.26 (0.21)	3.24 (0.98)	0.11 (0.08)
Enteritidis	95-2876	USDA APHIS (NVSL)	N	0.46 (0.41)	1.98 (1.71)	0.62 (0.36)
Enteritidis		FSIS	N	0.43 (0.36)	4.84 (1.97)	0.14 (0.11)
Infantis	890665	USMARC (urinary bladder)	N	0.91 (0.93)	4.17 (1.29)	0.16 (0.09)
Infantis	**CS-44**	**USMARC (cattle hide)**	**N**	**0.27 (0.07)**	**3.08 (1.32)**	**0.37 (0.09)**
Kentucky	94-6327	USDA APHIS (NVSL)	N	0.27 (0.25)	1.80 (1.52)	0.46 (0.10)
Kentucky	O1-B4	USMARC (lymph node)	N	0.34 (0.21)	0.60 (0.64)	0.27 (0.17)
Kiambu	CS-90	USMARC (cattle hide)	N	0.22 (0.10)	0.34 (0.09)	0.38 (0.07)
Mbandaka	O1-B7	USMARC (lymph node)	N	0.31 (0.23)	1.28 (0.67)	0.26 (0.28)
Meleagridis	NS-128	USMARC (cattle hide)	N	0.32 (0.04)	0.45 (0.21)	0.24 (0.09)
Montevideo	94-6529	USDA APHIS (NVSL)	N	0.34 (0.30)	1.82 (1.52)	0.64 (0.28)
Montevideo	**CS-80**	**USMARC (cattle hide)**	**N**	**0.08 (0.07)**	**1.04 (0.48)**	**0.13 (0.22)**
Montevideo	O1-A7	USMARC (lymph node)	N	0.24 (0.13)	1.03 (0.31)	0.29 (0.20)
Muenchen	CS-76	USMARC (cattle hide)	N	0.16 (0.12)	1.08 (0.58)	0.42 (0.18)
Muenchen	CS-93	USMARC (cattle hide)	N	0.20 (0.06)	1.02 (0.29)	0.05 (0.10)
Muenster	NS-145	USMARC (soil)	N	0.32 (0.38)	3.02 (2.06)	0.21 (0.10)
Muenster	BAA-1575	ATCC	N	0.47 (0.42)	3.16 (2.11)	0.20 (0.06)
Newport	644 AB2	USMARC	N	0.38 (0.30)	2.56 (1.41)	0.26 (0.22)
Newport	15124 PRH2	USMARC (preevis)	N	0.53 (0.38)	1.61 (1.19)	0.56 (0.30)
Newport		FSIS	N	0.35 (0.04)	2.83 (1.49)	0.35 (0.04)
Typhimurium	14218 PRB1	USMARC (cattle preevis)	N	0.36 (0.04)	2.89 (1.17)	0.16 (0.01)
Typhimurium	**14249 PRB1**	**USMARC (cattle preevis)**	**N**	**0.23 (0.14)**	**0.57 (0.44)**	**0.19 (0.04)**
Typhimurium	14164 PRB1	USMARC (cattle preevis)	N	0.36 (0.03)	3.22 (1.12)	0.48 (0.13)
Typhimurium	MHM 112 (ATCC 14028)	U. of Florida (avirulent)	N	0.27 (0.28)	2.26 (1.75)	0.22 (0.19)
Typhimurium	ATCC 14028	USMARC	N	0.27 (0.33)	2.25 (1.57)	0.25 (0.23)
Typhimurium	ATCC 19589	USMARC	N	1.06 (0.07)	1.08 (3.35)	1.45 (0.20)
Typhimurium	XLT4RV	USMARC (purge)	N	0.34 (0.13)	0.55 (3.38)	0.76 (0.09)
Non-ABR overall avg				***0.34 (0.30) A***	***1.83 (1.69) A***	***0.42 (0.31) B***
Anatum	**NS-127**	**USMARC (cattle hide)**	**Y (CSSUT)**	**0.15 (0.03)**	**1.55 (1.13)**	**0.59 (0.36)**
California		**USMARC**	**Y (Na)**	**0.35 (0.12)**	**0.37 (0.15)**	**0.70 (0.48)**
Dublin	NS-2	USMARC (cattle hide)	Y (CCpNaSSuT)	0.17 (0.06)	3.27 (3.33)	0.22 (0.18)
Dublin	CS-144	USMARC (cattle feces)	Y (AmpApFCfAxCSSuT)	0.13 (0.05)	0.66 (0.29)	0.67 (0.47)
Dublin	O8-G7	USMARC (lymph node)	Y (AmApFTAx)	0.15 (0.07)	1.12 (0.55)	0.35 (0.36)
Kiambu	CS-35	USMARC (cattle hide)	Y (AmApFCfAx)	0.13 (0.08)	0.28 (0.07)	0.48 (0.02)
Kiambu	CS-94	USMARC (cattle hide)	Y (SuT)	0.33 (0.07)	0.63 (0.39)	0.87 (0.38)
Meleagridis	NS-99	USMARC (cattle hide)	Y (CSSUT)	0.23 (0.11)	0.48 (0.26)	0.27 (0.30)
Meleagridis	NS-40	USMARC (cattle hide)	Y (AmApFCfAxCSSuT)	0.28 (0.14)	0.89 (0.55)	0.57 (0.26)
Montevideo	NS-82	USMARC (soil)	Y (AmApFCfAxCSSuT)	0.30 (0.15)	2.00 (1.66)	0.43 (0.14)
Montevideo	NS-83	USMARC (soil)	Y (CSSuT)	0.26 (0.07)	1.11 (0.73)	0.25 (0.31)
Montevideo	NS-3	USMARC (cattle hide)	Y (NaT)	0.52 (0.28)	3.80 (1.47)	1.13 (0.52)
Montevideo	NS-146	USMARC (cattle hide)	Y (T)	1.18 (0.94)	3.82 (1.68)	0.52 (0.16)
Montevideo	CS-2	USMARC (cattle hide)	Y (AmApFCfAxCSSuT)	0.55 (0.46)	5.17 (1.71)	1.67 (0.42)
Montevideo	**CS-77**	**USMARC (cattle hide)**	**Y (SuT)**	**0.10 (0.09)**	**1.28 (0.46)**	**0.40 (0.31)**
Muenchen	CS-15	USMARC (cattle hide)	Y (AmApFCfAxCSSuT)	0.11 (0.02)	1.17 (0.39)	0.53 (0.15)
Muenchen	CS-126	USMARC (cattle feces)	Y (SuT)	0.12 (0.13)	1.17 (0.62)	0.31 (0.21)
Muenchen	CS-146	USMARC (cattle feces)	Y (AmApFCfAxCSSuT)	0.12 (0.08)	1.10 (0.43)	0.68 (0.22)

TABLE 1. *Continued*

Salmonella	Designation	Source[b]	ABR[c]	Log reduction (CFU/mL)[d] LA	PAA	CPC
Newport	13109 PRB1	USMARC (preevis)	Y (AmApFTAxCGKSSuTe)	0.46 (0.37)	1.95 (0.84)	0.46 (0.62)
Newport	13212 PRH2	USMARC (preevis)	Y (CKSSuT)	0.32 (0.23)	1.83 (0.70)	0.68 (0.35)
Newport	13324 POH2	USMARC (cattle postinterv)	Y (AmApFTAxCSSuTe)	1.20 (0.34)	4.10 (0.75)	2.80 (0.16)
Newport	**NS-14**	**USMARC (cattle hide)**	**Y (AmApFCfAxCSSuT)**	**0.18 (0.03)**	**0.88 (0.63)**	**0.46 (0.18)**
Newport	O1-E2	USMARC (lymph node)	Y (AmpC)	0.18 (0.13)	1.77 (0.59)	0.25 (0.34)
Saint Paul	CS-1	USMARC (cattle hide)	Y (ApAzCCpGNaT)	0.30 (0.37)	2.14 (1.51)	0.08 (0.11)
Typhimurium	LT2	USMARC	Y (Na)	0.41 (0.17)	2.39 (0.80)	0.55 (0.14)
Typhimurium	720 AB2	USMARC	Y (CSSuTe)	0.56 (0.16)	5.19 (1.81)	0.44 (0.08)
Typhimurium	11241 PRB1	USMARC (cattle preevis)	Y (AmApFTAxCKSSuTe)	1.29 (0.29)	1.29 (0.29)	1.78 (0.24)
Typhimurium	12246 PRH2	USMARC (cattle preevis)	Y (AmApFTAxCKSSuTe)	1.30 (0.62)	1.78 (0.24)	0.35 (0.14)
Typhimurium	DT-104	USMARC (cattle)	Y (ACSSuTe)	0.52 (0.15)	3.41 (1.25)	0.12 (0.05)
Typhimurium	**O8-G4**	**USMARC (lymph node)**	**Y (AmApFTAx)**	**0.29 (0.15)**	**0.79 (0.57)**	**0.52 (0.37)**
Typhimurium	MHM 108 (ATCC 14028)	U. of Florida (avirulent)	Y (K)	0.38 (0.43)	2.28 (1.84)	0.35 (0.45)
Wentworth		USMARC	Y (Na)	0.59 (0.14)	2.85 (1.06)	1.16 (0.18)
ABR overall avg				***0.40 (0.41) A***	***1.94 (1.61) A***	***0.64 (0.59) A***

[a] ABR, antibiotic resistance. Antimicrobials used for developing resistant and/or tolerant *Salmonella*: LA, lactic acid (2%); PAA, peracetic acid (200 ppm); CPC, cetylpyridinium chloride (0.4%). Boldface indicates the selected strains for the challenge study based on numbers of antimicrobial compounds that reduced the *Salmonella* population less of each isolate, source of isolates, and strains frequently isolated from the processing plants.

[b] USMARC, U.S. Meat Animal Research Center; USDA, U.S. Department of Agriculture; APHIS, Animal and Plant Health Inspection Service; NVSL, National Veterinary Services Laboratories; FSIS, Food Safety and Inspection Service; ATCC, American Type Culture Collection; preevis, preevisceration carcasses; postinterv, postintervention carcasses.

[c] N, no; Y, yes. Resistance profiles: Ap, ampicillin; C, chloramphenicol; S streptomycin; Su, sulfonamide; Te, tetracycline; Tm, trimethoprim; Az, azithromycin; Tig, tigecycline; Cf, cefepime; Na, nalidixic acid; Cp, ciprofloxacin; Gm, gentamycin; P, penicillin; RA, rifampin; Am, amoxicillin–clavulanic acid; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulfisoxazole.

[d] Numbers in parentheses indicate standard deviations. Within the same treatment, means with a common letter are not significantly different ($P \geq 0.05$).

purge after mixing with each antimicrobial compound was 20%. A 100-μL aliquot of each treated isolate mixture was neutralized with 900 μL of Dey-Engley neutralizing broth (Difco, BD) supplemented with 35 g/L K_2HPO_4 (Sigma-Aldrich) and was serially 10-fold diluted with maximum recovery diluent (Difco, BD). Appropriate dilutions were spiral plated on TSA without glucose (Difco, BD) supplemented with 6.8 g/L sodium thiosulfate and 0.8 g/L ferric ammonium citrate (Sigma-Aldrich) for *Salmonella* enumeration. Plates were incubated at 37°C for 24 to 36 h before counting. For *Salmonella* enumeration, the black colonies were counted and compared with the black colonies from initial populations (0 s). TSA was used for enumeration during screening for antimicrobial tolerance to prevent the inhibitory effect of selective agents from XLD medium on the cells injured by the antimicrobial interventions.

Fresh beef and inocula preparations. Thirty-six fresh beef flanks (cutaneous trunci muscle) were collected from a local beef cattle processing plant, vacuum packaged, and stored at –20°C until use. Frozen beef flanks were thawed at 4°C, and each flank was marked into 16 sections (25-cm^2 [5 by 5-cm]) using a 100-cm^2 template (10 by 10 cm), sterile cotton swabs, and branding ink (Koch Supplies, Riverside, MO).

Two inocula (A and B) were prepared for the challenge study from selected *Salmonella* isolates that were previously identified for resistance to and/or tolerance of antimicrobials (Table 1). Isolates were selected for the challenge study based on the following combinations: numbers of antimicrobials that reduced the *Salmonella* population less (high level of tolerance), source of isolates, and strains frequently isolated from processing plants (Table 1). If high-tolerance isolates can be inactivated by the antimicrobial interventions, then the low-tolerance isolates should be easy to inactivate without overestimation. This demonstrates the efficacy of the antimicrobials under a worst-case scenario, partially resistant *Salmonella*. During cattle processing, beef carcasses are usually contaminated with diverse strains of the same species *(1)*. Therefore, each inoculum consisted of a mixture of selected isolates to simulate natural contamination. Each selected isolate was grown and adjusted for cell concentration as described above. For each inoculum, an equal volume of each strain was mixed using a vortexer and was 10-fold diluted with sterile saline solution. Inoculum A was a mixture of six selected strains of antibiotic-susceptible *Salmonella* (Agona NS-125, Agona CS-120, Cerro O1-A4, Infantis CS-44, Montevideo CS-80, and Typhimurium 14249 PRB1). Inoculum B was a mixture of six selected strains of ABR *Salmonella* (Anatum NS-127, California, Dublin CS-144, Montevideo CS-77, Newport NS-14, and Typhimurium O8-G4). The inocula were placed in an ice bath to prevent bacterial growth before inoculation on surfaces of fresh beef.

Inoculation, antimicrobial interventions, and sample collection. Each day of the challenge study, four marked beef flanks were divided into two groups. The surfaces of the first group of two beef flanks was inoculated with inoculum A and the second group of another two beef flanks was inoculated with inoculum B to a final concentration of approximately 10^4 to 10^5 CFU/cm^2, which could be detected during enumeration. Inoculat-

ed beef flanks were held at room temperature for 15 to 20 min to allow bacterial cells to attach to the surfaces of the fresh beef. LA and PAA were chosen for this study because these two compounds are commonly applied as antimicrobial interventions in beef processing. Although CPC is used in poultry processing, it may provide the foundation for disinfectant formulations that can improve the microbiological quality and safety of meat products and, thus, was included in the present study.

The inoculated fresh beef flanks (pH 5.6 to 5.8) were sprayed with (i) 4% LA (pH 2.1), (ii) 400 ppm of PAA (pH 4.3), or (iii) 0.8% CPC (pH 6.1) for 15 s at 20 lb/in^2 and at 22 to 25°C using an USMARC model spray wash cabinet *(24)* with three oscillating spray nozzles (SS5010, Spray Systems Co., Wheaton, IL) at 60 cycles per min and a flow rate of 6.8 L/min. The distance between nozzles and the surface of the meat tissue was 17 cm. Eight tissue samples (25-cm^2) before (initial population) and after spraying antimicrobial compounds (total 16 samples) were randomly collected by excision and placed individually into filter bags (Whirl-Pak, Nasco, Ft. Atkinson, WI). All bags containing tissue samples were held for 10 min at room temperature before enumeration.

Enumeration and culturing. The tissue samples were neutralized with 75 mL of Dey-Engley neutralizing broth (Difco, BD) supplemented with 35 g/L K_2HPO_4 (Sigma-Aldrich) and homogenized for 2 min using a stomacher (BagMixer 400, Interscience, Weymouth, MA), and then each homogenate was 10-fold serially diluted with maximum recovery diluent. Appropriate dilutions were spiral plated (Spiral Biotech, Norwood, MA) using TSA (Difco, BD) plates for enumeration of aerobic bacteria, whereas XLD agar plates (Oxoid, Remel Inc., Lenexa, KS) were used for *Salmonella* enumeration. After agar plates were incubated at 37°C for 24 to 36 h, aerobic bacteria and *Salmonella* were counted. The limit of detection using a spiral plater was 80 CFU/cm^2.

Statistical analyses. Colony counts were transformed to log CFU/cm^2 values from three experimental replications of each antimicrobial treatments × eight tissue sections × two inocula ($n = 48$). 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 the Tukey-Kramer test method with the probability level at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Thirty-three ABR and 35 non-ABR *Salmonella* isolates were screened with half-strength LA (2%), PAA (200 ppm), or CPC (0.4%) to determine which isolates had resistance or tolerance to the tested antimicrobials. The sources of isolates and sensitivity and resistance of each isolate to LA (2%), PAA (200 ppm), and CPC (0.4%) are presented in Table 1. LA (2%), PAA (200 ppm), and CPC (0.4%) reduced non-ABR *Salmonella* in beef purge by 0.16 to 1.06, 0.25 to 4.84, and 0.05 to 1.45 log, respectively. The same antimicrobial compounds reduced ABR *Salmonella* by 0.10 to 1.30, 0.28 to 5.19, and 0.08 to 2.80 log, respectively. The reduction data for non-ABR *Salmonella* were pooled together regardless of strains to compare with pooled reduction data from ABR *Salmonella* to determine the inhibitory effect of LA (2%), PAA (200 ppm), and CPC (0.4%) in beef purge systems. There was no difference ($P \geq 0.05$) in reduction between non-ABR and ABR isolates treated with LA and PAA, but reductions differed after treatment with CPC ($P \leq 0.05$; Table 1). Overall, within the same species, each selected strain responded to antimicrobials differently. For non-ABR *Salmonella,* LA, PAA, and CPC reduced Anatum strain APHIS 94-6411 by 0.33, 0.55, and 0.84 log, respectively, whereas LA, PAA, and CPC reduced Anatum strain CS-45 by 0.52, 3.87, and 0.85 log, respectively (Table 1). Similarly, for ABR *Salmonella,* LA, PAA, and CPC reduced Typhimurium strain 720 AB2 by 0.56, 5.19, and 0.44 log, whereas the same concentrations of antimicrobial compounds reduced Typhimurium strain O8-G4 by 0.29, 0.79, and 0.52 log, respectively. This indicates that the sensitivity and resistance of the *Salmonella* isolates depended on the antimicrobial compound used and was strain specific. Kwon and Ricke *(30)* reported that the sensitivity and resistance of Typhimurium to organic acids varied by acid type and concentration, whereas the sensitivity and resistance of *Salmonella* to antimicrobials depended on the strains *(35)*.

For the challenge study, a six-strain mixture of non-ABR *Salmonella* (Agona NS-125, Agona CS-120, Cerro O1-A4, Infantis CS-44, Montevideo CS-80, and Typhimurium 14249 PRB1) and a six-strain mixture of ABR *Salmonella* (Anatum NS-127, California, Dublin CS-144, Montevideo CS-77, Newport NS-14, and Typhimurium O8-G4) from Table 1 were selected for inoculation on surfaces of fresh beef.

Fresh beef flanks inoculated with *Salmonella* were challenged with antimicrobial compounds. The data were pooled regardless of non-ABR or ABR *Salmonella* to determine the efficacy of antimicrobial compounds. As shown in Figure 1, LA (4%), CPC (0.8%), and PAA (400 ppm) reduced the *Salmonella* population on fresh beef surfaces by 2.04, 1.38, and 1.34 log CFU/cm^2, respectively. The inactivation effect of LA was greater ($P \leq 0.05$) than that of CPC or PAA; the inactivation effects of CPC and PAA were the same ($P \geq 0.05$). The reductions of *Salmonella* on fresh beef surfaces using LA, PAA, and CPC found in the present study were similar to those of previous reports *(12, 23)*. Kalchayanand et al. *(23)* inoculated fresh beef flanks with *Salmonella* Typhimurium and *Salmonella* Newport; after treatment with 4% LA or 200 ppm of PAA, they found *Salmonella* reductions of 3.1 and 0.9 log CFU/cm^2, respectively. In another study, in which lean and adipose tissues that were inoculated with *Salmonella* Typhimurium were subjected to 1% CPC spray treatment *(12), Salmonella* Typhimurium populations on both tissues were immediately reduced by more than 2.5 log CFU/cm^2 *(12)*. This reduction of *Salmonella* with 1% CPC is higher than was found in the current study, likely due to the high pressure (125 lb/in^2) spray wash in combination with higher CPC temperature (35°C). In the present study, LA, CPC, and PAA reduced aerobic bacteria on surfaces of fresh beef by 1.42, 1.34, and 1.23 log CFU/cm^2, respectively. LA reduced aerobic bacteria more ($P \leq 0.05$) than PAA but had the same inactivation effect ($P \geq 0.05$) as CPC. The aerobic bacterial reductions agreed with those found in the previous report *(23),* 1.9 and 1.0 log

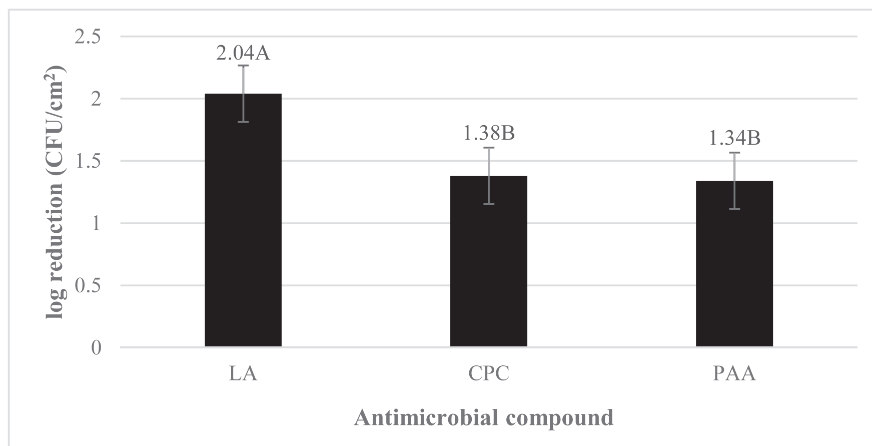

FIGURE 1. *Evaluation of antimicrobial compounds against* Salmonella *on surface of fresh beef. LA, lactic acid (4%); CPC, cetylpyridinium chloride (0.8%); PAA, peracetic acid (400 ppm).Within a treatment, means with a common letter are not significantly different (*$P \geq 0.05$*).*

CFU/cm^2, when 4% LA and 200 ppm of PAA were applied to the fresh beef flanks, respectively.

In the current study, the reductions of antimicrobial-tolerant non-ABR and antimicrobial-tolerant ABR *Salmonella* isolates on fresh beef tissue surfaces were determined after treatment with LA, CPC, and PAA. Numerically, all three antimicrobial compounds had greater reductions of ABR than non-ABR *Salmonella* (Table 2). However, these differences were not statistically ($P \geq 0.05$) different.

LA can inhibit the growth of many types of food spoilage bacteria, including gram-negative species of *Enterobacteriaceae* and *Pseudomonadaceae (14)*. Several studies indicated that LA effectively reduced *Salmonella* on surfaces of fresh beef, which was consistent with findings in the current study. LA spray treatment reduced *Salmonella* by 1.2 to 1.8 log CFU/cm^2 *(2, 17, 22)*. The variation in sensitivity to LA or other organic acids is strain dependent and may vary depending on physiological status of the organism and the physicochemical characteristics of the external environment *(38)*. Although our findings that LA applied to inoculated fresh beef reduced *Salmonella* by 1.95 and 2.14 log are higher than in the previous report *(2),* there was no significant difference in the reduction of non-ABR and ABR *Salmonella* (Table 2).

In the United States, PAA used as an antimicrobial intervention in beef processing facilities is commonly applied to beef carcasses and products at a maximum concentration of 400 ppm *(41)*. Efficacy of PAA has been variable in reducing STEC and *Salmonella* contamination on several beef products *(18, 27, 28)*. A study by Kocharunchitt et al. in 2020 *(29)* noted that PAA ($\geq$200 ppm) reduced *Salmonella* $\geq$2.6 log on surfaces of beef carcasses when applied during the spray chilling process. However, the reductions of *Salmonella* on hot carcass surfaces were 0.7 log *(27)*. In another study, PAA (200 ppm) reduced non-ABR and ABR *Salmonella* Newport by 0.6 and 0.7 log CFU/cm^2, and non-ABR and ABR *Salmonella* Typhimurium by 0.5 and 0.7 log CFU/cm^2, respectively *(18)*. The results reported herein indicate that PAA (400 ppm) reduced *Salmonella* on surfaces of fresh beef by 1.22 to 1.45 log CFU/cm^2. Furthermore, Geornaras et al. *(18)* also indicated that ABR strains of *Salmonella* Newport and *Salmonella* Typhimurium were as sensitive to the PAA treatments as were the non-ABR strains of these serotypes. Our current results agree with those of Geornaras et al. *(18)* that PAA was equally effective when applied to antimicrobial-tolerant non-ABR and to antimicrobial-tolerant ABR *Salmonella*. Treatment with PAA (200 ppm) resulted in the same ($P \geq 0.05$) overall average reduction of non-ABR *Salmonella* as of ABR *Salmonella* (Table 1). This indicates that the strain selection for the challenge study was suitable. The greater log reductions found in the present study may possibly be due to differences in PAA concentration (400 ppm in this study, 200 ppm in Geornaras et al. *(18)*) and in method of application.

CPC is a quaternary ammonium and is used as an antimicrobial agent to treat poultry carcasses or parts at a concentration level not to exceed 0.8% by weight *(42)*. Several studies have demonstrated that CPC reduces *Salmonella* Typhimurium and prevents cross-contamination

TABLE 2. *Effect of antimicrobial compounds on non-ABR and ABR* Salmonella *inoculated fresh beef*[a]

			Log reduction (CFU/cm^2)	
Treatment	*n*	ABR	Aerobic bacteria	*Salmonella*
LA	48	N	1.38 ± 0.48 A	1.95 ± 0.68 A
LA	48	Y	1.46 ± 0.56 A	2.14 ± 1.07 A
CPC	48	N	1.28 ± 0.31 A	1.33 ± 0.39 B
CPC	48	Y	1.38 ± 0.63 A	1.43 ± 0.33 B
PAA	48	N	1.15 ± 0.45 B	1.22 ± 0.48 B
PAA	48	Y	1.31 ± 0.46 A	1.45 ± 0.42 B

[a] Treatments: LA, lactic acid (4%); CPC, cetylpyridinium chloride (0.8%); PAA, peracetic acid (400 ppm). ABR, antibiotic resistance; N, no; Y, yes. Within the same treatment, means with a common letter are not significantly different ($P \geq 0.05$).

(7, 26, 45). CPC has not been approved for use in beef processing because it leaves excessive residue for human consumption. However, CPC provides the foundation for disinfectant formulations that can improve the microbiological quality and safety of meat products. The study of CPC to control bacteria during beef cattle processing is limited. Because of the CPC residue, a further study of the effect of beef carcass spray chill with water on the residue may need to be investigated. Spray application with 1% CPC reduced aerobic bacteria and *Enterobacteriaceae* by 2 and 1 log CFU/100 cm^2, respectively *(6)*. CPC (1%) spray treatment reduced *Salmonella* on contaminated lean and adipose tissues by approximately 5 log CFU/cm^2 *(12)*. The large reduction may possibly be due to high pressure spray treatment, which physically removed *Salmonella* Typhimurium from lean and adipose tissues. In another study, 0.5% CPC reduced *Salmonella* Typhimurium in ground beef by 0.7 log CFU/g when it was applied to contaminated beef trimmings before grinding *(36)*. Direct comparison of the results obtained in the current study with the results of previous studies is difficult due to differences in experimental parameters (e.g., antimicrobial concentrations tested, treatment application parameters, target populations and species, muscle tissue type).

The *Salmonella* cell counts reported in this study were enumerated for untreated and treated cells using selective medium. Selective medium was used to reduce background flora, which can reach 10^5 CFU/100 cm^2 on preevisceration carcasses *(33)* and may interfere with *Salmonella* enumeration. Antimicrobial treatments not only kill but can also inflict sublethal injury to microorganisms. Therefore, use of selective medium for enumeration may lead to overestimation of the effectiveness of antimicrobial compounds, because sublethally injured cells may not recover and grow in the presence of selective agents. In the present study, both controls and treated samples were enumerated on a nonselective medium (TSA) to allow injured cells to resuscitate and multiply. The efficacy of all three antimicrobial compounds tested for aerobic bacteria resulted in reductions of more than 1 log CFU/cm^2 (Table 2), which indicated that these antimicrobial compounds were effective for use as antimicrobial interventions. No single intervention technology can provide 100% assurance of food product safety. Systems that provide a microbial reduction of at least 1 log unit would be considered to provide appropriate improvement in the microbiological status of the product *(25)*.

More information is needed to determine whether ABR bacteria are more resistant to most antimicrobial compounds currently used and which compound is the most effective in reducing ABR *Salmonella*. The results of this study indicate that treatment with LA and PAA, currently in place in fresh meat processing facilities, and with CPC (approved for poultry processing) are equally effective in reducing non-ABR and ABR *Salmonella* that may be present during processing. Furthermore, the development and use of resistant and/or tolerant strains of *Salmonella* for testing in this experiment ensures that the efficacy of LA, PAA, and CPC in reducing ABR *Salmonella* is not overestimated. This means the antimicrobial treatments tested were still effective under a worst-case scenario, against *Salmonella* selected to be partially resistant to the treatments tested. The findings also indicate that LA is the most effective in reducing resistant and/or tolerant strains of ABR and non-ABR *Salmonella* on surfaces of fresh beef; PAA and CPC are equally effective but less effective than LA.

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