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Sensitivity of Shiga Toxin–Producing *Escherichia coli,* Multidrug-Resistant *Salmonella,* and Antibiotic-Susceptible *Salmonella* to Lactic Acid on Inoculated Beef Trimmings

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ABSTRACT

Studies were performed to determine whether lactic acid treatments used to reduce *Escherichia coli* O157:H7 on beef trimmings are also effective in controlling non-O157 Shiga toxin–producing *E. coli* (nSTEC), and multidrug-resistant and antibiotic-susceptible *Salmonella*. Beef trimming pieces (10 by 5 by 1 cm) were inoculated (3 log CFU/cm^2) separately with four-strain mixtures of rifampin-resistant *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145. Similarly, in a second study, trimmings were separately inoculated with rifampin-resistant *E. coli* O157:H7, and antibiotic-susceptible or multidrug-resistant (MDR and/or MDR-AmpC) *Salmonella* Newport and *Salmonella* Typhimurium. Inoculated trimmings were left untreated (control) or were immersed for 30 s in 5% lactic acid solutions (25 or 55°C). No differences ($P \geq 0.05$) were obtained among surviving counts of *E. coli* O157:H7 and those of the tested nSTEC serogroups on lactic acid–treated (25 or 55°C) samples. Counts (3.1 to 3.3 log CFU/cm^2) of *E. coli* O157:H7 and nSTEC were reduced ($P < 0.05$) by 0.5 to 0.9 (25°C lactic acid) and 1.0 to 1.4 (55°C lactic acid) log CFU/cm^2. Surviving counts of *Salmonella* on treated trimmings were not influenced by serotype or antibiotic resistance phenotype and were similar ($P \geq 0.05$) or lower ($P < 0.05$) than surviving counts of *E. coli* O157:H7. Counts (3.0 to 3.3 log CFU/cm^2) were reduced ($P < 0.05$) by 0.5 to 0.8 (*E. coli* O157:H7) and 1.3 to 1.5 (*Salmonella*) log CFU/cm^2 after treatment of samples with 25°C lactic acid. Corresponding reductions following treatment with lactic acid at 55°C were 1.2 to 1.5 (*E. coli* O157:H7) and 1.6 to 1.9 (*Salmonella*) log CFU/cm^2. Overall, the results indicated that lactic acid treatments used against *E. coli* O157:H7 on beef trimmings should be similarly or more effective against the six nSTEC serogroups and against multidrug-resistant and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium.

There are a reported 300 to 400 known serotypes of Shiga toxin–producing *Escherichia coli*; however, not all have been linked to or have the potential to cause human illness *(16, 23, 30, 41)*. The subset of Shiga toxin–producing *E. coli* (STEC) strains that have been clinically associated with bloody diarrhea are designated as enterohemorrhagic *E. coli* (EHEC). As such, all EHEC strains are considered pathogenic, but not all STEC are capable of causing human illness *(16, 30, 41)*. The O157:H7 serotype of EHEC is an important cause of human illness from beef products in the United States, and as such, until recently, has been the primary control target of the research community and regulators *(16, 18, 19, 30)*. In terms of research, numerous studies have reported on the prevalence of *E. coli* O157:H7 on or in cattle hides, feces, carcass surfaces, and resulting beef products *(8, 17, 18, 23, 24)*, and on physical and chemical decontamination interventions to reduce contamination levels of this pathogen at various stages of the beef production chain *(18, 29, 34, 36)*. From the regulatory perspective, the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA-FSIS) has considered *E. coli* O157:H7 an adulterant in raw, nonintact beef products and components of such products (e.g., beef manufacturing trimmings) since the 1990s *(39)*.

In addition to EHEC O157:H7, several non-O157 EHEC strains have also been associated with human illness *(7, 16, 19, 30, 41)*. The clinical manifestations of infection with some non-O157 EHEC can be as severe as those resulting from infection with EHEC O157:H7 *(7, 26, 30)*. According to the Centers for Disease Control and Prevention, there were 451 laboratory-confirmed cases of non-O157 EHEC infection in 2010, and the six most commonly isolated serogroups were O26, O45, O103, O111, O121, and O145 *(12)*. By comparison, 442 laboratory-confirmed cases of EHEC O157 infection occurred during the same year *(12)*. To date, there has only been one non-O157 EHEC outbreak definitively linked to beef in the United States *(38)*. It occurred in 2010, involved three case patients, and was traced to ground beef contaminated with EHEC O26. Other foods that have been associated with non-O157 EHEC outbreaks in the United States include apple cider, milk, punch, blueberries, strawberries, and lettuce-based salads *(7, 11, 30, 41)*.

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Cattle, and other ruminants, are natural reservoirs of non-O157 Shiga toxin–producing *E. coli* (nSTEC), as is the case for *E. coli* O157:H7 *(16, 18, 23, 24)*. The prevalence of nSTEC on beef cattle hides, carcasses during processing, beef trim, and ground beef has been reported by Arthur et al. *(1),* Barkocy-Gallagher et al. *(3),* Bosilevac and Koohmaraie *(6),* Bosilevac et al. *(4),* Hussein *(23),* and Hussein and Bollinger *(24)*. nSTEC strains were isolated from 10 to 30% of samples of boneless beef trim destined for grinding *(4),* and from 7.3% of ground beef samples collected from 18 U.S. commercial producers *(6)*. Recent concerns on the public health significance of nSTEC contamination in beef products has resulted in the USDA-FSIS declaring nSTEC serogroups O26, O45, O103, O111, O121, and O145 as adulterants in raw, nonintact beef products and components of such products *(39)*. As of June 2012, the USDA-FSIS implemented a sampling and testing program for these nSTEC serogroups in beef manufacturing trimmings *(39, 40)*.

Salmonella contamination in beef products is also considered a public health concern, particularly if the strain is resistant to multiple antimicrobial drugs *(35)*. Infection with an antibiotic-resistant strain presents a challenge for treatment of the clinical disease *(2, 25)*. A study by Bosilevac et al. *(5)* found 4.2% of 4,136 ground beef samples from U.S. commercial producers positive for *Salmonella,* and furthermore, 0.6% of the samples contained strains that were resistant to two or more antibiotics. Undercooked ground beef contaminated with multidrug-resistant (MDR or MDR-AmpC) strains of *Salmonella* Newport and *Salmonella* Typhimurium has been implicated in several human illness outbreaks *(13, 33, 37)*. The MDR phenotype is defined as resistance to at least ampicillin, chloramphenicol, streptomycin, sulfamethoxazole-sulfisoxazole, and tetracycline (ACSSuT); and the MDR-AmpC phenotype is defined as resistance to at least ACSSuT, amoxicillin–clavulanic acid, and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC of ≥ 2 μg/ml) *(10, 20)*. A concern associated with *Salmonella* strains with resistance to multiple antibiotics is whether their antibiotic resistance properties also make them less susceptible to chemical decontamination interventions applied during beef processing *(2)*.

Lactic acid is one of the most commonly used antimicrobials for decontamination of carcasses, cuts, and beef trimmings in U.S. beef processing plants, and numerous inoculated challenge studies *(2, 9, 14, 15, 18, 21, 22, 28, 32)* have reported on the decontamination efficacy of this organic acid against *E. coli* O157:H7. In view of the recent attention to nSTEC, studies are needed to determine whether lactic acid treatments used to control *E. coli* O157:H7 contamination on fresh beef products are also effective against the nSTEC serogroups of current concern. Furthermore, although it has been demonstrated that *Salmonella* contamination on beef carcasses and cuts is also reduced by lactic acid treatments *(2, 9, 14, 21, 28),* studies comparing the efficacy of this antimicrobial agent against multidrug-resistant and antibiotic-susceptible *Salmonella* inocula, in the same study, are limited. Therefore, the objective of this study was to compare the efficacy of 5% lactic acid, applied at 25 or 55°C, against *E. coli* O157:H7 (as the control pathogen), nSTEC serogroups O26, O45, O103, O111, O121, and O145, and multidrug-resistant and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium. The nSTEC and *Salmonella* inocula were evaluated in separate studies.

MATERIALS AND METHODS

Bacterial strains and preparation of inocula. The tested pathogen inocula were each composed of four strains (Tables 1 and 2). The *E. coli* O157:H7 strains were from our laboratory's culture collection, while the nSTEC strains belonging to serogroups O26, O45, O103, O111, O121, and O145 were kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, Pennsylvania State University, University Park), Dr. Pina Fratamico (Eastern Regional Research Center, USDA–Agricultural Research Service [ARS], Wyndmoor, PA), and Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE) (Table 1). To allow for selective enumeration of the STEC inocula from the natural microbiota associated with fresh beef, rifampin-resistant (100 μg/ml) variants of the strains were used for inoculation of samples. Rifampin-resistant cultures of the *E. coli* O157:H7 strains were already available in our culture collection; while, for the nSTEC strains, spontaneous rifampin-resistant variants were selected according to the method described by Kaspar and Tamplin *(27)*.

The *Salmonella* Newport (antibiotic-susceptible and MDR-AmpC phenotypes) and *Salmonella* Typhimurium (antibiotic-susceptible, MDR, and MDR-AmpC phenotypes) strains used in the study were kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD) (Table 2) and were all hydrogen sulfide producers, as indicated by the formation of black-centered colonies on xylose lysine deoxycholate (XLD) agar (Acumedia, Lansing, MI). The antibiotic resistance phenotype of the *Salmonella* serotype strains was confirmed with the CMV2AGNF panel of the Sensititre antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH) per the manufacturer's instructions. Strains with an MDR phenotype were resistant to at least ACSSuT; and strains with an MDR-AmpC phenotype were resistant to at least ACSSuT, amoxicillin–clavulanic acid, and ceftiofur, and had an decreased susceptibility to ceftriaxone (MIC of ≥ 2 μg/ml) *(10, 20)*.

The rifampin-resistant STEC strains were individually cultured (35°C, 20 to 24 h) in tryptic soy broth (TSB; Difco, BD, Sparks, MD) supplemented with rifampin (100 μg/ml; Sigma-Aldrich, St. Louis, MO) (TSB + rif), while the *Salmonella* serotype strains were individually cultured (35°C, 20 to 24 h) in TSB. Broth cultures were subcultured once by transferring a 0.1-ml aliquot of the first broth culture into 10 ml of fresh TSB + rif or TSB and incubating at 35°C for 20 to 24 h. Cells of individual cultures (10 ml) were harvested by centrifugation (4,629 × *g* [6,000 rpm] at 4°C for 15 min; Eppendorf model 5810 R, Hamburg, Germany), washed with 10 ml of phosphate-buffered saline (PBS; pH 7.4; 0.2 g/liter KH_2PO_4, 1.5 g/liter $Na_2HPO_4 \cdot 7H_2O$, 8.0 g/liter NaCl, and 0.2 g/liter KCl) and centrifuged again. Resulting washed cell pellets were resuspended in 10 ml of PBS, and cell suspensions of the four strains belonging to the same *E. coli* serotype or serogroup, or *Salmonella* Newport or *Salmonella* Typhimurium antibiotic resistance phenotype, were then combined and serially diluted in PBS to a final concentration of approximately 6 log CFU/ml.

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TABLE 1. *Shiga toxin–producing* E. coli *strains used in the study*

E. coli serotype/serogroup	Strain	Origin	Source[a]
O26:H11	hSTEC_03	Human	USMARC
O26:H2	93.0494	Human	*E. coli* Reference Center
O26	0.1302	Cow	*E. coli* Reference Center
O26:H11	5.2217	Human	*E. coli* Reference Center
O45	99E_2750	Human	USMARC
O45	O45-2	Human	USMARC
O45:H2	05-6545	Human	ERRC
O45:H2	96-3285	Human	ERRC
O103	MDR0089	Beef	USMARC
O103:H2	87.1368	Goat	*E. coli* Reference Center
O103:H2	90.1764	Cow	*E. coli* Reference Center
O103:H2	92.0084	Human	*E. coli* Reference Center
O111:H8	hSTEC_08	Human	USMARC
O111	93.0523	Human	*E. coli* Reference Center
O111	4.0005	Cow	*E. coli* Reference Center
O111	4.0522	Cow	*E. coli* Reference Center
O121	10896	Human	USMARC
O121	imp_450	Beef	USMARC
O121:H19	97-3068	Human	ERRC
O121:NM	03-4064	Human	ERRC
O145:NM	hSTEC_22	Human	USMARC
O145	MAY109	Beef	USMARC
O145:NM	03-4699	Human	ERRC
O145:H18	07865	Cow	ERRC
O157:H7	ATCC 43895	Raw hamburger	ATCC
O157:H7	C1-057	Bovine feces	Carlson et al. *(8)*
O157:H7	C1-072	Bovine feces	Carlson et al. *(8)*
O157:H7	C1-109	Bovine feces	Carlson et al. *(8)*

[a] USMARC, U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE; *E. coli* Reference Center, Pennsylvania State University, University Park, PA; ERRC, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA; ATCC, American Type Culture Collection.

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Inoculation and decontamination of beef trimmings. Beef trimmings samples for inoculation were fabricated from beef chuck rolls purchased from a slaughter facility in northern Colorado. The chuck rolls (from carcasses fabricated approximately 48 h postharvest) were collected directly from the production line of the facility and were vacuum packaged before being transported to the Department of Animal Sciences at Colorado State University. On arrival at the laboratory (within 1 h of collection), the meat cuts were stored at 4°C and used within 48 h.

Beef trimmings samples (10 by 5 by 1 cm [length by width by thickness]) weighing approximately 100 g were cut from the chuck rolls and inoculated with one of the tested pathogen inocula to a target level of approximately 3 log CFU/cm^2. Specifically, 0.1 ml of the specific pathogen inoculum was randomly distributed, with a micropipette (approximately 10 μl per drop), over the surface of one flat side of the beef samples. After a 10-min attachment period (4°C), samples were turned over and inoculated on the second flat side in the same way.

As previously indicated, the decontamination efficacy of the lactic acid treatments against the nSTEC and *Salmonella* inocula was evaluated in separate studies, with *E. coli* O157:H7 included in both studies as the control pathogen. For all tested pathogen inocula, inoculated beef trimmings were either left untreated (control) or were treated with 5% lactic acid (pH 2.16 $\pm$ 0.06; Purac America, Lincolnshire, IL) at 25 or 55°C. The lactic acid treatments were applied by completely immersing individual samples, for 30 s, in 150 ml of the solution in a sterile bag (19 by 30 cm; Whirl-Pak, Nasco, Modesto, CA). Fresh solutions were used for treatment of each sample. After treatment, samples were drained for 60 s (30 s per side) in a strainer, placed in sterile sample filter bags (19 by 30 cm; Whirl-Pak, Nasco), and held for 1 h (4°C) before analysis for surviving bacterial cells. The 1-h period simulated the potential time lapse between collection of treated beef trim samples from the production floor of a grinding facility and their subsequent microbial analysis in a laboratory. In the present study, beef samples were weighed before and after lactic acid treatment (i.e., after the 60-s draining period) for determination of moisture pickup in decontaminated samples.

Microbiological and pH analyses. A 100-ml aliquot of Dey/Engley neutralizing broth (Difco, BD) was added to individual untreated (control) or treated beef samples in the sample filter bags and then homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Meat homogenates were serially diluted (0.1% buffered peptone water; Difco, BD), and appropriate dilutions were surface plated (0.1 ml of diluted samples, or 1 ml [distributed over three plates] of undiluted samples) on different media, depending on the study, for enumeration of inoculated pathogen populations. In the study with the nSTEC inocula, all samples were plated on tryptic soy agar (TSA; Acumedia) supplemented with rifampin (100 μg/ml) (TSA+rif). In the study with the *Salmonella* inocula, samples inoculated with rifampin-resistant *E. coli* O157:H7 (as the control pathogen) were plated on TSA+rif and modified sorbitol MacConkey agar (mSMAC; MacConkey sorbitol agar [Difco, BD] supplemented with 2.5 mg/liter potassium tellurite [Sigma-Aldrich] and 20 mg/liter novobiocin [Sigma-Aldrich]), while samples

TABLE 2. Salmonella *Newport and* Salmonella *Typhimurium strains used in the study*

Salmonella serotype	Strain	Antibiotic resistance phenotype	Origin	Source[a]
Newport	FSL S5-639	Susceptible	Human	Cornell
Newport	CVM N4505	Susceptible	Ground turkey	CVM/FDA
Newport	CVM N18445	Susceptible	Ground beef	CVM/FDA
Newport	CVM N1509	Susceptible	Ground turkey	CVM/FDA
Newport	FSL S5-436	MDR-AmpC	Bovine	Cornell
Newport	FSL S5-920	MDR-AmpC	Bovine	Cornell
Newport	CVM 22698	MDR-AmpC	Pork chop	CVM/FDA
Newport	CVM N19852	MDR-AmpC	Ground beef	CVM/FDA
Typhimurium	FSL S5-536	Susceptible	Human	Cornell
Typhimurium	CVM N7300	Susceptible	Chicken breast	CVM/FDA
Typhimurium var. O:5− (Copenhagen)	CVM N15788	Susceptible	Ground beef	CVM/FDA
Typhimurium var. O:5− (Copenhagen)	CVM N18534	Susceptible	Chicken breast	CVM/FDA
Typhimurium	FSL R6-215	MDR	Human	Cornell
Typhimurium	FSL R8-2540	MDR	Human	Cornell
Typhimurium	CVM N6431	MDR	Chicken breast	CVM/FDA
Typhimurium var. O:5− (Copenhagen)	CVM 30662	MDR	Chicken breast	CVM/FDA
Typhimurium var. Copenhagen	FSL S5-786	MDR-AmpC	Bovine	Cornell
Typhimurium var. O:5− (Copenhagen)	CVM N176	MDR-AmpC	Chicken breast	CVM/FDA
Typhimurium	CVM 33831	MDR-AmpC	Cattle	CVM/FDA
Typhimurium var. O:5− (Copenhagen)	CVM 30034	MDR-AmpC	Ground turkey	CVM/FDA

[a] Cornell, Department of Food Science, Cornell University, Ithaca, NY; CVM/FDA, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD.

inoculated with any of the tested *Salmonella* inocula were plated on XLD agar. All samples from both studies were also plated on TSA for enumeration of total bacterial populations. TSA+rif, mSMAC, and XLD agar plates were incubated at 35°C for 24 h, and TSA plates at 25°C for 72 h, before counting of colonies. Uninoculated beef samples were also analyzed for total bacterial counts (TSA), and for any naturally present rifampin-resistant (TSA+rif), sorbitol-negative (mSMAC), and hydrogen sulfide–producing (XLD agar) microbial populations. The detection limit of the microbiological analysis was 1 CFU/cm^2 and was calculated by considering the lowest dilution plated (i.e., the undiluted sample), the volume of the lowest dilution plated (i.e., 1 ml), the volume of Dey/Engley neutralizing broth added to each sample (i.e., 100 ml), and the surface area of the inoculated trimmings (i.e., 100 cm^2).

For pH measurements, uninoculated, untreated and lactic acid–treated (25 or 55°C) beef samples were homogenized (2 min; Masticator) with distilled water (1:1 ratio of sample weight to volume of water), and the pH of the resulting homogenate was measured with a Denver Instruments (Arvada, CO) pH meter fitted with a glass electrode. An additional set of untreated and treated samples was stored at 4°C, in Whirl-Pak bags, and pH measurements were taken after 24 h, as previously described.

Statistical analysis. Three repetitions (using meat from different production days, and new cultures and lactic acid solutions) were conducted for the study with the nSTEC inocula and two repetitions for the study with the *Salmonella* inocula; within each repetition, three untreated and three treated (lactic acid at 25 or 55°C) samples were analyzed per inoculum. Each repetition was considered as a blocking factor in a complete randomized block design. Microbial counts were transformed into log CFU per square centimeter before statistical analysis. ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation, using the PROC MIXED command of SAS (version 9.3, SAS Institute Inc., Cary, NC), were used to determine whether statistical differences existed among surviving counts of the nSTEC serogroups and *E. coli* O157:H7, or among surviving counts of the *Salmonella* Newport and *Salmonella* Typhimurium antibiotic resistance phenotypes and *E. coli* O157:H7. In addition to this analysis, a Tukey-adjusted ANOVA test, using the PROC GLM command of SAS, was used to compare counts of untreated and treated (25 or 55°C lactic acid) samples within each *E. coli* serotype or serogroup or *Salmonella* Newport and *Salmonella* Typhimurium antibiotic resistance phenotype. The pH data were analyzed with a Student's t test (PROC GLM). In all cases, P values less than 0.05 ($P < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

pH and moisture pickup of trimmings. Untreated beef trimmings had initial pH values of 5.41 ± 0.10 and 5.19 ± 0.17 in the studies conducted with the nSTEC and *Salmonella* inocula, respectively (Table 3). As expected, immersion (30 s) of beef samples in solutions of 5% lactic acid lowered ($P < 0.05$) the pH of trimmings. More specifically, pH values of lactic acid–treated trimmings were 0.96 to 1.38 pH units lower ($P < 0.05$) than the pH of the untreated controls; however, no ($P \geq 0.05$) differences in pH were obtained among samples treated with 25 or 55°C solutions, within each study. An additional set of samples held for 24 h at 4°C was also measured for pH to determine whether there would be any appreciable changes to the initial pH (i.e., 1 h after treatment) of decontaminated samples. Changes in pH after 24-h storage of lactic acid–treated trimmings ranged from 0.14 to 0.37 pH unit increases. Overall, pH values of treated samples after 24 h at 4°C were 0.54 to 1.21 pH units lower ($P < 0.05$) than the

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TABLE 3. *The pH of untreated and treated beef trimmings after 1 and 24 h at 4°C*[a]

Study	Treatment	1 h after treatment	24 h after treatment
nSTEC inocula	Untreated control	5.41 ± 0.10 a A	5.44 ± 0.28 a A
	Lactic acid (25°C)	4.12 ± 0.21 b A	4.26 ± 0.26 b A
	Lactic acid (55°C)	4.03 ± 0.16 b A	4.23 ± 0.24 b A
Salmonella inocula	Untreated control	5.19 ± 0.17 a A	5.14 ± 0.08 a A
	Lactic acid (25°C)	4.22 ± 0.21 b B	4.53 ± 0.20 b A
	Lactic acid (55°C)	4.23 ± 0.09 b B	4.60 ± 0.12 b A

[a] pH is expressed as mean ± standard deviation. Treatment was with 5% lactic acid at either 25 or 55°C. nSTEC, non-O157 Shiga toxin–producing *E. coli*. Within a column and within each study, means with a common lowercase letter are not different ($P \geq 0.05$); within a row, means with a common uppercase letter are not different ($P \geq 0.05$).

pH of the corresponding untreated controls included in each study. The moisture pickup of all lactic acid–treated samples was 5.7% ± 1.6% (25°C solution) and 5.9% ± 2.0% (55°C solution) in the study with the nSTEC inocula, and 5.7% ± 1.6% (25°C solution) and 5.9% ± 2.8% (55°C solution) in the study with the *Salmonella* inocula (data not shown in tables). Grinding facilities considering using immersion as the method for treating beef trim with lactic acid would have to consider the exposure time to the solution and its effect on the moisture content of the product, and any potential regulatory requirements for labeling of such product.

Efficacy of decontamination treatments against *E. coli* O157:H7 and nSTEC serogroups. The total bacterial count of the uninoculated beef samples was 3.4 ± 0.4 log CFU/cm^2, and no naturally present rifampin-resistant microbial populations were detected (detection limit: 1 CFU/cm^2) on TSA+rif (data not shown in tables). Decontamination of inoculated trimmings with 5% lactic acid reduced ($P < 0.05$) initial pathogen counts (3.1 to 3.3 log CFU/cm^2) by 0.5 to 0.9 (25°C lactic acid) and 1.0 to 1.4 (55°C lactic acid) log CFU/cm^2, irrespective of STEC inoculum (Table 4). Similarly, total bacterial counts (3.5 to 3.6 log CFU/cm^2) were reduced ($P < 0.05$) by 0.7 to 1.1 (25°C lactic acid) and 1.0 to 1.4 (55°C lactic acid) log CFU/cm^2 (Table 4). Bacterial counts (total and pathogen) of samples treated with lactic acid at 55°C were numerically, but not always statistically, lower than those of samples treated with 25°C solutions. Previous studies on the efficacy of lactic acid against *E. coli* O157:H7 on beef tissue samples include a wide range of experimental procedures relative to the starting levels of the pathogen, the concentration and temperature of the solution, the method of application, the exposure time, the type of beef tissue treated, and the culture media used for enumeration of surviving populations. In general, however, *E. coli* O157:H7 reductions of approximately 1 to 3 log units have been reported for lactic acid–treated beef samples *(2, 14, 15, 21, 22, 28, 32)*.

In all cases, in the present study, surviving counts of *E. coli* O157:H7 on treated samples were not ($P \geq 0.05$) different from those of any of the tested nSTEC inocula (Table 4). Therefore, lactic acid decontamination (25 or 55°C) of beef trimmings was equally effective against *E. coli* O157:H7 and all six nSTEC serogroups. Furthermore, among the nSTEC inocula, no ($P \geq 0.05$) differences in surviving counts on treated trimmings were generally obtained. In the one exception, the mean count of *E. coli* O103 on samples treated with 25°C lactic acid was statistically ($P < 0.05$) higher than the counts of *E. coli* O26, O121, and O145. Numerically, however, the *E. coli* O103 count was only 0.3 log units higher than those of *E. coli* O26, O121, and O145. Such a small log unit difference is not considered as biologically significant *(31)*. A study by Cutter and Rivera-Betancourt *(14)* also demonstrated that a lactic acid treatment (2%, 35°C, 15-s spray) was similarly

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TABLE 4. *Populations of rifampin-resistant* E. coli *O157:H7 and six non-O157 Shiga toxin–producing* E. coli *serogroups (recovered with TSA+rif), and total bacteria (recovered with TSA), on untreated and treated beef trimmings*[a]

E. coli serotype/ serogroup	TSA+rif			TSA		
	Untreated control	Lactic acid (25°C)	Lactic acid (55°C)	Untreated control	Lactic acid (25°C)	Lactic acid (55°C)
O157:H7	3.2 ± 0.1 a A	2.5 ± 0.2 ab B	1.8 ± 0.4 a C	3.6 ± 0.4 a A	2.8 ± 0.3 a B	2.3 ± 0.5 a B
O26	3.2 ± 0.1 a A	2.4 ± 0.1 b B	2.1 ± 0.3 a C	3.5 ± 0.2 a A	2.8 ± 0.3 a B	2.4 ± 0.6 a B
O45	3.2 ± 0.1 a A	2.5 ± 0.2 ab B	1.8 ± 0.5 a C	3.6 ± 0.2 a A	2.8 ± 0.4 a B	2.2 ± 0.5 a C
O103	3.2 ± 0.1 a A	2.7 ± 0.2 a B	2.1 ± 0.3 a C	3.5 ± 0.1 a A	2.8 ± 0.2 a B	2.4 ± 0.3 a C
O111	3.2 ± 0.1 a A	2.5 ± 0.1 ab B	2.2 ± 0.3 a B	3.6 ± 0.5 a A	2.5 ± 0.6 a B	2.3 ± 0.5 a B
O121	3.1 ± 0.1 a A	2.4 ± 0.2 b B	2.0 ± 0.2 a C	3.6 ± 0.4 a A	2.9 ± 0.4 a B	2.5 ± 0.5 a B
O145	3.3 ± 0.4 a A	2.4 ± 0.3 b B	2.2 ± 0.3 a B	3.6 ± 0.2 a A	2.7 ± 0.5 a B	2.6 ± 0.6 a B

[a] Populations are expressed as mean ± standard deviation in log CFU per square centimeter. Treatment was with 5% lactic acid at either 25 or 55°C. TSA+rif, tryptic soy agar supplemented with rifampin (100 μg/ml); TSA, tryptic soy agar. Within a column, means with a common lowercase letter are not different ($P \geq 0.05$); within a row and within each culture medium, means with a common uppercase letter are not different ($P \geq 0.05$).

TABLE 5. *Populations of rifampin-resistant* E. coli *O157:H7 (recovered with TSA+rif or mSMAC), antibiotic-susceptible and multidrug-resistant (MDR and/or MDR-AmpC)* Salmonella *Newport and* Salmonella *Typhimurium (recovered with XLD agar), and total bacteria (recovered with TSA), on untreated and treated beef trimmings*[a]

Inoculum	TSA+rif (*E. coli* O157:H7) and XLD agar (*Salmonella*)			mSMAC (*E. coli* O157:H7) and XLD agar (*Salmonella*)			TSA		
	Untreated control	Lactic acid (25°C)	Lactic acid (55°C)	Untreated control	Lactic acid (25°C)	Lactic acid (55°C)	Untreated control	Lactic acid (25°C)	Lactic acid (55°C)
E. coli O157:H7	3.2 ± 0.1 a A	2.7 ± 0.1 a B	2.0 ± 0.4 a C	3.0 ± 0.1 a A	2.2 ± 0.0 a B	1.5 ± 0.5 a C	4.0 ± 0.3 a A	3.2 ± 0.2 a B	3.0 ± 0.3 a B
Salmonella Newport susceptible	3.1 ± 0.0 a A	1.8 ± 0.1 bc B	1.5 ± 0.4 ab B	3.1 ± 0.0 a A	1.8 ± 0.1 bc B	1.5 ± 0.4 a B	3.9 ± 0.4 a A	3.0 ± 0.2 a B	2.7 ± 0.4 a B
Salmonella Newport MDR-AmpC	3.1 ± 0.1 a A	1.6 ± 0.2 c B	1.2 ± 0.3 b C	3.1 ± 0.1 a A	1.6 ± 0.2 c B	1.2 ± 0.3 a C	4.1 ± 0.3 a A	2.8 ± 0.3 a B	2.6 ± 0.3 a B
Salmonella Typhimurium susceptible	3.2 ± 0.1 a A	1.9 ± 0.2 b B	1.6 ± 0.4 ab B	3.2 ± 0.1 a A	1.9 ± 0.2 b B	1.6 ± 0.4 a B	4.2 ± 0.5 a A	3.1 ± 0.4 a B	2.5 ± 0.4 a C
Salmonella Typhimurium MDR	3.3 ± 0.0 a A	2.0 ± 0.2 b B	1.4 ± 0.5 ab C	3.3 ± 0.0 a A	2.0 ± 0.2 ab B	1.4 ± 0.5 a C	3.9 ± 0.1 a A	3.0 ± 0.2 a B	2.5 ± 0.4 a C
Salmonella Typhimurium MDR-AmpC	3.0 ± 0.1 a A	1.7 ± 0.2 bc B	1.3 ± 0.4 b B	3.0 ± 0.1 a A	1.7 ± 0.2 bc B	1.3 ± 0.4 a B	3.9 ± 0.3 a A	3.1 ± 0.4 a B	2.7 ± 0.4 a B

[a] Populations are expressed as mean ± standard deviation in log CFU per square centimeter. Treatment was with 5% lactic acid at either 25 or 55°C. TSA+rif, tryptic soy agar supplemented with rifampin (100 μg/ml); mSMAC, modified sorbitol MacConkey agar; XLD, xylose lysine deoxycholate; TSA, tryptic soy agar. Within a column, means with a common lowercase letter are not different ($P \geq 0.05$); within a row and within each culture medium, means with a common uppercase letter are not different ($P \geq 0.05$).

effective against *E. coli* O157:H7 and two nSTEC serotypes (O26:H11 and O111:H8).

Efficacy of decontamination treatments against *E. coli* O157:H7 and against multidrug-resistant and susceptible *Salmonella* Newport and *Salmonella* Typhimurium. The natural microbial contamination level of the beef samples used in this study was 3.9 ± 0.4 log CFU/cm^2 (data not shown in tables). Background rifampin-resistant (on TSA+rif), sorbitol-negative (on mSMAC), and hydrogen sulfide–producing (on XLD agar) microbial populations were not detected (<1 CFU/cm^2) in the uninoculated samples analyzed.

Counts of inoculated *E. coli* O157:H7 on untreated and treated trimmings were recovered with TSA+rif as well as mSMAC in this study because of concerns about comparing *E. coli* O157:H7 counts recovered with a rifampin-supplemented nonselective medium (TSA+rif) with those of the *Salmonella* inocula, which were recovered with a selective medium (XLD agar). More specifically, sublethally injured *E. coli* O157:H7 cells, due to exposure to 25 or 55°C lactic acid, might have recovered on TSA+rif (resulting in higher surviving counts), while sublethally injured *Salmonella* cells might have not recovered on XLD agar (resulting in lower surviving counts). For this reason, *E. coli* O157:H7–inoculated samples were also plated on the selective medium, mSMAC, and separate statistical comparisons were conducted with the *E. coli* O157:H7 counts recovered with TSA+rif and mSMAC, and those of the *Salmonella* inocula (Table 5).

E. coli O157:H7 counts (3.2 and 3.0 log CFU/cm^2 on TSA+rif and mSMAC, respectively) were reduced ($P < 0.05$) by 0.5 (TSA+rif) and 0.8 (mSMAC) log CFU/cm^2 after treatment of trimmings with 25°C lactic acid solutions, and by 1.2 (TSA+rif) and 1.5 (mSMAC) log CFU/cm^2 after treatment with 55°C lactic acid solutions (Table 5). Irrespective of *Salmonella* inoculum, decontamination of beef samples reduced ($P < 0.05$) counts (3.0 to 3.3 log CFU/cm^2) by 1.3 to 1.5 (25°C lactic acid) and 1.6 to 1.9 (55°C lactic acid) log CFU/cm^2. The reductions obtained for the *Salmonella* inocula are within the approximately 1 to 3 log unit range of reductions reported by previous studies that evaluated the decontamination efficacy of lactic acid against *Salmonella* under various testing parameters *(2, 9, 14, 21, 28)*. In the present study, total bacterial counts (3.9 to 4.2 log CFU/cm^2) were reduced ($P < 0.05$) by 0.8 to 1.3 (25°C lactic acid) and 1.0 to 1.7 (55°C lactic acid) log CFU/cm^2. Similar to the study with the nSTEC inocula, surviving counts (total, *E. coli* O157:H7, and *Salmonella*) of samples treated with 55°C lactic acid solutions were numerically, but not always statistically, lower than those of samples treated with 25°C solutions (Table 5).

Overall, surviving counts of *E. coli* O157:H7, recovered with TSA+rif or mSMAC, on lactic acid–treated samples were similar ($P \geq 0.05$) or higher ($P < 0.05$) than those of the multidrug-resistant and susceptible *Salmonella* Newport and *Salmonella* Typhimurium inocula (Table 5). Therefore, the tested lactic acid treatments were equally or more effective against the *Salmonella* inocula than they

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were against *E. coli* O157:H7. Within the tested *Salmonella* inocula, similar ($P \geq 0.05$) surviving counts were generally obtained on all treated samples, irrespective of *Salmonella* serotype (Newport and Typhimurium) or antibiotic resistance phenotype (susceptible, MDR, and/or MDR-AmpC). In one exception, the mean count of the MDR-AmpC phenotype of *Salmonella* Newport on 25°C lactic acid–treated samples was lower ($P < 0.05$) than the counts of the antibiotic-susceptible and MDR phenotypes of *Salmonella* Typhimurium; however, the numerical difference (0.3 to 0.4 log CFU/cm^2) between the counts of these inocula is not considered biologically significant. In a similar study by Arthur et al. *(2)*, it was reported that reductions obtained for multidrug-resistant *Salmonella* Newport and *Salmonella* Typhimurium on beef tissue samples treated with various acid and nonacid decontamination treatments (lactic acid, acetic acid, FreshFX, hot water, electrolyzed water, and ozone) were similar to or higher than the reductions obtained for *E. coli* O157:H7 and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium. In this particular study, 2% lactic acid, applied by spraying (25 psi, 20 s), reduced all tested pathogen inocula by 1.1 to 1.8 log CFU/cm^2 *(2)*.

In conclusion, the results indicated that lactic acid immersion treatments (5%, applied at 25 or 55°C) used against *E. coli* O157:H7 on beef trimmings should be similarly effective against the six nSTEC serogroups of current concern, and similarly or more effective against multidrug-resistant and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium. Thus, based on these findings, meat processors that have already validated lactic acid immersion treatments against *E. coli* O157:H7, under the conditions tested, should not have to conduct separate studies to validate the treatments against nSTEC serogroups O26, O45, O103, O111, O121, and O145, and *Salmonella*. Overall, the results of this study provide evidence that the effect of lactic acid decontamination treatments should be similar against *E. coli* O157:H7 as well as other vegetative pathogens of enteric origin that may be present on fresh beef trimmings.

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