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Efficacy of Chemical Interventions against *Escherichia coli* O157:H7 and Multidrug-Resistant and Antibiotic-Susceptible *Salmonella* on Inoculated Beef Trimmings

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ABSTRACT

Studies were conducted to compare the decontamination efficacy of six chemical treatments against *Escherichia coli* O157:H7 and multidrug-resistant and antibiotic-susceptible *Salmonella* inoculated on beef trimmings. The inocula, comprising four-strain mixtures of rifampin-resistant *E. coli* O157:H7 and antibiotic-susceptible or multidrug-resistant (MDR and/or MDR-AmpC) *Salmonella* Newport and *Salmonella* Typhimurium, were inoculated (3 log CFU/cm^2) separately onto samples (10 by 5 by 1 cm) derived from beef chuck rolls. Samples were left untreated (control), were immersed for 30 s in acidified sodium chlorite (0.1%, pH 2.5), peroxyacetic acid (0.02%, pH 3.8), sodium metasilicate (4%, pH 12.6), Bromitize Plus (0.0225% active bromine, pH 6.6), or AFTEC 3000 (pH 1.2), or were immersed for 5 s in SYNTRx 3300 (pH 1.0). Levels of surviving *Salmonella* on treated trimmings were not influenced by serotype or antibiotic resistance phenotype and were generally similar ($P \geq 0.05$) or lower ($P < 0.05$) than levels of surviving *E. coli* O157:H7 regardless of antimicrobial treatment. Overall, depending on chemical treatment (reductions within each chemical treatment were similar among all tested inocula), initial counts of *E. coli* O157:H7 (2.7 to 3.1 log CFU/cm^2) were reduced ($P < 0.05$) by 0.2 to 1.4 log CFU/cm^2. Similarly, initial counts of the tested *Salmonella* inocula (2.8 to 3.3 log CFU/cm^2) were reduced ($P < 0.05$) by 0.4 to 1.4 (*Salmonella* Newport, antibiotic susceptible), 0.3 to 1.4 (*Salmonella* Newport, MDR-AmpC), 0.2 to 1.5 (*Salmonella* Typhimurium, antibiotic susceptible), 0.4 to 1.3 (*Salmonella* Typhimurium, MDR), and 0.4 to 1.5 (*Salmonella* Typhimurium, MDR-AmpC) log CFU/cm^2, depending on antimicrobial treatment. Reductions obtained with sodium metasilicate were 1.3 to 1.5 log CFU/cm^2, regardless of inoculum, and reductions obtained with the five remaining antimicrobial treatments were 0.2 to 0.7 log CFU/cm^2 (depending on treatment). Findings of this study should be useful to regulatory authorities and the meat industry as they consider *Salmonella* contamination on beef trimmings.

Decontamination interventions comprising physical and/or chemical treatments have been developed and are implemented by beef processing plants in the United States to minimize the potential for involvement of undercooked ground beef in infections by pathogens such as *Escherichia coli* O157:H7 *(21)*. Another pathogen of concern in ground beef is *Salmonella (10, 19, 23)*. A recent concern with *Salmonella* is the ability of multidrug-resistant strains to cause infection *(10, 14, 15, 19, 23, 26)*. A 2011 salmonellosis outbreak was linked to ground beef contaminated with a *Salmonella* Typhimurium strain resistant to eight antibiotics (amoxicillin–clavulanic acid, ampicillin, ceftriaxone, cefoxitin, kanamycin, streptomycin, sulfisoxazole, and tetracycline); it caused 20 illnesses in seven U.S. states, and among 17 patients that were interviewed, 8 (47%) were hospitalized *(10)*.

Studies investigating the prevalence and antibiotic susceptibility of *Salmonella* from cattle hides, carcasses, and fresh beef products have reported recovery of isolates of various *S. enterica* serotypes with resistance to two or more antibiotics *(3, 5, 7, 27, 29)*. The prevalence of *Salmonella* in six U.S. beef processing plants was reported to be 89.6% on hides, 50.2% on preevisceration carcasses (sampled after hide removal), and 0.8% on postintervention carcasses (sampled within 2 h of entering the chiller after having received the full complement of carcass decontamination interventions) *(7)*. In these plants, 16.7, 11.7, and 0.3% of the samples from cattle hides, preevisceration carcasses, and postintervention carcasses, respectively, were positive for *Salmonella* isolates with resistance to two or more antibiotics *(7)*. In another study *(5), Salmonella* was isolated from 4.2% of 4,136 ground beef samples collected from 18 commercial producers, and 0.6% of the samples contained strains that were resistant to 2 to 10 antibiotics.

Two *Salmonella* multidrug-resistance patterns of public health significance in the United States are (i) resistance to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT; hereinafter referred to as MDR) and (ii) resistance to at least ACSSuT, amoxicillin–clavulanic acid, and ceftiofur and a decreased susceptibility to ceftriaxone (MIC of ≥2 μg/ml) (hereinafter referred to as

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MDR-AmpC) *(9, 13, 27, 28)*. *Salmonella* Typhimurium strains with the MDR phenotype and *Salmonella* Newport strains with the MDR-AmpC phenotype have been associated with outbreaks of foodborne illness from ground beef in the United States *(23)*.

Studies have confirmed the effectiveness of physical and chemical decontamination interventions (e.g., steam vacuuming, hot or cold water and organic acid washing, and spray chilling) for reducing the prevalence of pathogens on beef carcasses *(1, 3, 4, 7)*. However, processing of carcasses into primals, subprimals, and trimmings provides an opportunity for recontamination of newly exposed meat surfaces *(12)*. Various chemical treatments have been evaluated for decontamination of beef trimmings before grinding, including lactic acid, acidified sodium chlorite, peroxyacetic acid, sodium metasilicate, potassium lactate, and cetylpyridinium chloride *(6, 11, 12, 17, 18, 22)*. The efficacy of these and other antimicrobials for reducing *E. coli* O157:H7 contamination on beef carcasses and/or trimmings has been established; however, in comparison, data on the antimicrobial effects of decontamination treatments against *Salmonella* are limited, especially on the efficacy of interventions against multidrug-resistant versus antibiotic-susceptible strains of *Salmonella*. Such data are needed to answer questions relative to the sensitivity of antibiotic- or multidrug-resistant *Salmonella* to chemical decontamination interventions applied during beef processing *(2)*.

Studies on the decontaminating efficacy of chemical interventions against antibiotic-sensitive and -resistant *Salmonella* are needed, especially relative to effectiveness against *E. coli* O157:H7. Thus, the objective of this study was to compare the antimicrobial effects of six chemical decontamination treatments for beef trimmings against *E. coli* O157:H7 and multidrug-resistant (MDR and/or MDR-AmpC) and antibotic-susceptible phenotypes of *Salmonella* Newport and *Salmonella* Typhimurium.

MATERIALS AND METHODS

Bacterial strains and preparation of inocula. The five *Salmonella* inocula prepared for this study included antibiotic-susceptible and MDR-AmpC phenotypes of *Salmonella* Newport and antibiotic-susceptible, MDR, and MDR-AmpC phenotypes of *Salmonella* Typhimurium. Each inoculum was a mixture of four strains. The *Salmonella* Newport and *Salmonella* Typhimurium strains (kindly provided by Dr. Martin Wiedmann, Department of Food Science, Cornell University, Ithaca, NY; and Dr. Shaohua Zhao, U.S. Food and Drug Administration, Center for Veterinary Medicine, Laurel, MD) (Table 1) were all hydrogen sulfide producers, as indicated by the formation of black-centered colonies on xylose lysine deoxycholate (XLD) agar (Acumedia, Lansing, MI). The *E. coli* O157:H7 inoculum also consisted of four strains: rifampin-resistant (100 μg/ml) variants of strains ATCC 43895, C1-057, C1-072, and C1-109 (C1 strains were of bovine fecal origin) previously used in beef-related studies *(8, 20)*.

Antibiotic resistance phenotypes of the *Salmonella* Newport and *Salmonella* Typhimurium strains (Table 1) were confirmed with the CMV2AGNF panel of the Sensititre antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH). MICs were determined for ampicillin, amoxicillin–clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole in accordance with the manufacturer's instructions. Results were interpreted based on available breakpoints *(25)*. Azithromycin was also included on the test panel, but breakpoints were not available for this antibiotic; therefore, results for this antimicrobial agent are not included in Table 1. *Salmonella* Typhimurium strains with the MDR phenotype were resistant to at least ACSSuT, and *Salmonella* Newport and *Salmonella* Typhimurium strains with the MDR-AmpC phenotype were resistant to at least ACSSuT, amoxicillin–clavulanic acid, and ceftiofur and had a decreased susceptibility to ceftriaxone (MIC $\geq$ 2 μg/ml) *(9, 13)*.

Working cultures of the *Salmonella* Newport and *Salmonella* Typhimurium strains were maintained on tryptic soy agar (TSA; Acumedia), and those of the rifampin-resistant *E. coli* O157:H7 strains were maintained on TSA supplemented with 100 μg/ml rifampin (Sigma-Aldrich, St. Louis, MO) (TSA + rif). Before each experiment, a single colony of each *Salmonella* and *E. coli* O157:H7 strain was separately inoculated into 10 ml of tryptic soy broth (TSB; Difco, BD, Sparks, MD) or TSB supplemented with rifampin (100 μg/ml) (TSB + rif), respectively. After incubation at 35°C for 20 to 24 h, broth cultures were subcultured (0.1 ml) into 10 ml of fresh TSB or TSB + rif and incubated at 35°C for 20 to 24 h. Cell cultures of the four *E. coli* O157:H7 strains or the four strains belonging to the same *Salmonella* Newport or *Salmonella* Typhimurium antibiotic resistance phenotype were then combined and harvested by centrifugation (4,629 $\times$ *g*, 15 min, 4°C; model 5810 R, Eppendorf, Hamburg, Germany). Cell pellets were 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), centrifuged again, and resuspended in 40 ml of PBS. Cell suspensions were serially diluted in PBS to a final level of approximately 6 log CFU/ml.

Inoculation of beef trimmings. Beef trimming samples were fabricated from fresh (approximately 48 h postslaughter) beef chuck rolls purchased from a slaughter facility in northern Colorado. The beef cuts were collected directly from the production line before the application of any chemical decontamination treatment and were vacuum packaged and transported to the Department of Animal Sciences at Colorado State University. Upon arrival within 1 h of collection, the beef cuts were either used immediately or stored at 4°C and used within 48 h. Beef trimming samples 10 by 5 by 1 cm (length by width by thickness) and weighing approximately 100 g were cut from the chuck rolls and spot inoculated with one of the pathogen inocula to a target level of approximately 3 log CFU/cm^2. More specifically, an aliquot (0.1 ml) of the inoculum was randomly distributed (approximately 10 μl per drop) over the surface of one flat side of the meat samples; after a 10-min attachment period (4°C), the samples were turned over and inoculated on the second flat side in the same way.

Chemical decontamination of beef trimmings. Solutions of the tested antimicrobial treatments were prepared according to the manufacturers' instructions and were applied at approved concentrations *(24)* or at a pH recommended by the manufacturer. The six treatments were evaluated in separate studies: (i) acidified sodium chlorite (0.1%, pH 2.5 $\pm$ 0.0; Sanova, Ecolab, St. Paul, MN), (ii) peroxyacetic acid (0.02%, pH 3.8 $\pm$ 0.1; Inspexx 200, Ecolab), (iii) sodium metasilicate (4%, pH 12.6 $\pm$ 0.1; AvGard XP, Danisco USA, New Century, KS), (iv) Bromitize Plus (0.0225% active bromine, pH 6.6 $\pm$ 0.1; Enviro Tech Chemical Services, Modesto, CA), (v) AFTEC 3000 (pH 1.2; Advanced Food

TABLE 1. Salmonella *Newport and* Salmonella *Typhimurium strains used in the study*

Salmonella serotype	Strain	Antibiotic resistance profile[a]	Resistance phenotype[b]	Origin	Source
Newport	FSL S5-639	S	Susceptible	Human	Cornell[c]
Newport	CVM N4505	S	Susceptible	Ground turkey	FDA-CVM[d]
Newport	CVM N18445	S	Susceptible	Ground beef	FDA-CVM
Newport	CVM N1509	S	Susceptible	Ground turkey	FDA-CVM
Newport	FSL S5-436	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Bovine	Cornell
Newport	FSL S5-920	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Bovine	Cornell
Newport	CVM 22698	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Pork chop	FDA-CVM
Newport	CVM N19852	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Ground beef	FDA-CVM
Typhimurium	FSL S5-536	S	Susceptible	Human	Cornell
Typhimurium	CVM N7300	S	Susceptible	Chicken breast	FDA-CVM
Typhimurium var. O:5− (Copenhagen)	CVM N15788	S	Susceptible	Ground beef	FDA-CVM
Typhimurium var. O:5− (Copenhagen)	CVM N18534	S	Susceptible	Chicken breast	FDA-CVM
Typhimurium	FSL R6-215	AMP, CHL, STR, FIS, TET	MDR	Human	Cornell
Typhimurium	FSL R8-2540	AMP, CHL, STR, FIS, TET	MDR	Human	Cornell
Typhimurium	CVM N6431	AMP, CHL, STR, FIS, TET	MDR	Chicken breast	FDA-CVM
Typhimurium var. O:5− (Copenhagen)	CVM 30662	AMP, CHL, STR, FIS, TET	MDR	Chicken breast	FDA-CVM
Typhimurium var. Copenhagen	FSL S5-786	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Bovine	Cornell
Typhimurium var. O:5− (Copenhagen)	CVM N176	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Chicken breast	FDA-CVM
Typhimurium	CVM 33831	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT, KAN	MDR-AmpC	Cattle	FDA-CVM
Typhimurium var. O:5− (Copenhagen)	CVM 30034	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, NAL, KAN	MDR-AmpC	Ground turkey	FDA-CVM

[a] S, sensitive to all tested antibiotics. Resistance profiles: AMP, ampicillin; CHL, chloramphenicol; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline; AUG2, amoxicillin–clavulanic acid; XNL, ceftiofur; AXO, ceftriaxone; FOX, cefoxitin; KAN, kanamycin; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid.

[b] MDR, resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT); MDR-AmpC, resistant to at least ACSSuT, amoxicillin–clavulanic acid, and ceftiofur and decreased susceptibility to ceftriaxone (MIC $\geq$ 2 μg/ml).

[c] Department of Food Science, Cornell University, Ithaca, NY.

[d] U.S. Food and Drug Administration, Center for Veterinary Medicine, Laurel, MD.

Technologies, Shreveport, LA), and (vi) SYNTRx 3300 (pH 1.0; Synergy Technologies, Shreveport, LA). In each study, inoculated trimmings were either left untreated (control) or were decontaminated by completely immersing individual samples in the test solution (150 ml, 25 $\pm$ 2°C) in a sterile Whirl-Pak bag (19 by 30 cm; Nasco, Modesto, CA). Samples were exposed to the antimicrobial treatment for 30 s, except for SYNTRx 3300, which was applied for 5 s as per the manufacturer's recommendation. Fresh solutions were used for treatment of each sample. After treatment, beef samples were allowed to drain in a strainer for 60 s (30 s per side) and then transferred to Whirl-Pak filter bags (19 by 30 cm). The moisture content of decontaminated samples was determined by weighing the trimmings before and after antimicrobial treatment (i.e., after the 60-s draining period).

Microbiological and pH analyses. Untreated (control) and treated trimmings were analyzed for inoculated pathogen populations and total bacterial counts. Decontaminated samples were held at 4°C for 1 h (after draining) before analysis for surviving bacterial cells; this holding 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. D/E neutralizing broth (100 ml; Difco, BD) was added to individual untreated or treated samples, which were then pummeled for 2 min (Masticator, IUL Instruments, Barcelona, Spain), and the resulting meat homogenate was serially diluted in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions of samples inoculated with *E. coli* O157:H7 were surface 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]) for determination of *E. coli* O157:H7 counts and on TSA for determination of total bacterial counts. Beef trimmings inoculated with any of the tested *Salmonella* inocula were surface plated on XLD agar (for *Salmonella* counts) and TSA (for total bacterial counts). Colonies were counted after incubation

TABLE 2. *pH of untreated and decontaminated beef trimmings*

Chemical treatment	pH[a]
Acidified sodium chlorite	5.75 ± 0.16 A
Untreated control	5.79 ± 0.11 A
Peroxyacetic acid	5.98 ± 0.14 A
Untreated control	6.04 ± 0.13 A
Sodium metasilicate	8.66 ± 0.55 A
Untreated control	6.04 ± 0.13 B
Bromitize Plus	5.68 ± 0.22 A
Untreated control	5.61 ± 0.09 A
AFTEC 3000	4.68 ± 0.23 B
Untreated control	5.47 ± 0.09 A
SYNTRx 3300	4.77 ± 0.24 B
Untreated control	5.47 ± 0.09 A

[a] Values are mean ± standard deviation, $n = 6$. Within each study (i.e., chemical treatment and corresponding untreated control), means with a common letter are not significantly different ($P \geq 0.05$).

of plates at 35°C for 24 h (TSA + rif, mSMAC, and XLD agar) or at 25°C for 72 h (TSA). Uninoculated samples of the meat used to conduct the studies were also analyzed for counts of the natural microflora (on TSA) and for any naturally present rifampin-resistant (on TSA + rif), sorbitol-negative (on mSMAC), and hydrogen sulfide–producing (on XLD agar) microbial populations. The detection limit of the microbiological analyses was 1 CFU/cm^2.

Uninoculated meat samples left untreated or treated with the tested antimicrobials were analyzed for pH. Decontaminated trimmings were held for 1 h at 4°C after treatment before pH analysis. Samples were homogenized (Masticator) for 2 min with distilled water (1:1 ratio of sample weight to volume of water), and the pH of the homogenate was measured with a pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO).

Statistical analysis. Each antimicrobial agent was evaluated independently; therefore, separate statistical analyses were performed for the tested inocula within each antimicrobial treatment. Two repetitions were conducted per chemical treatment, and within each repetition, three untreated and three treated samples were analyzed per inoculum treatment. Each repetition (using meat from different production days and new cultures and chemical solutions) was considered a blocking factor in a randomized complete block design. Analysis of variance procedures and 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 compare counts (transformed into log CFU per square centimeter) of surviving *E. coli* O157:H7 and the five *Salmonella* inocula on untreated and treated trimmings. A Student-based *t* test (PROC MIXED) was used to compare microbial counts of samples before (untreated control) and after antimicrobial treatment within each tested pathogen inoculum. The pH data were analyzed with a Student-based *t* test using the PROC GLM procedure of SAS. In all cases, *P* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

pH and moisture of trimmings. Untreated beef samples had a pH of 5.47 to 6.04 (Table 2). Immersion (30 s) of the trimmings in solutions of acidified sodium chlorite, peroxyacetic acid, or Bromitize Plus did not ($P \geq 0.05$) affect the initial (i.e., 1 h after treatment) pH of samples. However, compared with the pH of the untreated control included in each study, treatment with sodium metasilicate raised ($P < 0.05$) the initial pH of samples by 2.62 units, and treatment with AFTEC 3000 or SYNTRx 3300 lowered ($P < 0.05$) the pH of samples by 0.79 and 0.70 units, respectively. Limited data (three to six samples per treatment; not shown in Table 2) indicated that these significant effects to the initial pH of trimmings were less severe after 24 h of storage of samples at 4°C. After 24 h, the initial pH of sodium metasilicate–treated samples decreased from 8.66 to 6.52, and initial pH values of

TABLE 3. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with 0.1% acidified sodium chlorite*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA + rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	Acidified sodium chlorite	Untreated control	Acidified sodium chlorite
E. coli O157:H7	3.1 ± 0.0 a A	2.6 ± 0.1 a B	2.7 ± 0.1 c A	2.2 ± 0.2 c B
Salmonella Newport				
Susceptible	3.0 ± 0.1 ab A	2.6 ± 0.1 a B	3.0 ± 0.1 ab A	2.6 ± 0.1 a B
MDR-AmpC	3.1 ± 0.2 ab A	2.5 ± 0.1 ab B	3.1 ± 0.2 ab A	2.5 ± 0.1 ab B
Salmonella Typhimurium				
Susceptible	3.1 ± 0.1 a A	2.6 ± 0.3 a B	3.1 ± 0.1 a A	2.6 ± 0.3 a B
MDR	3.1 ± 0.1 a A	2.7 ± 0.1 a B	3.1 ± 0.1 a A	2.7 ± 0.1 a B
MDR-AmpC	2.9 ± 0.0 b A	2.3 ± 0.0 b B	2.9 ± 0.0 bc A	2.3 ± 0.0 bc B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA + rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

TABLE 4. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with 0.02% peroxyacetic acid*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA+rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	Peroxyacetic acid	Untreated control	Peroxyacetic acid
E. coli O157:H7	3.1 ± 0.0 b A	2.4 ± 0.1 b B	2.8 ± 0.1 c A	2.3 ± 0.2 c B
Salmonella Newport				
Susceptible	3.2 ± 0.1 b A	2.5 ± 0.2 ab B	3.2 ± 0.1 b A	2.5 ± 0.2 ab B
MDR-AmpC	3.0 ± 0.1 b A	2.4 ± 0.1 b B	3.0 ± 0.1 b A	2.4 ± 0.1 bc B
Salmonella Typhimurium				
Susceptible	3.1 ± 0.1 b A	2.6 ± 0.2 ab B	3.1 ± 0.1 b A	2.6 ± 0.2 a B
MDR	3.3 ± 0.1 a A	2.6 ± 0.3 a B	3.3 ± 0.1 a A	2.6 ± 0.3 a B
MDR-AmpC	3.1 ± 0.1 b A	2.5 ± 0.2 ab B	3.1 ± 0.1 b A	2.5 ± 0.2 ab B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA+rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

AFTEC 3000– or SYNTRx 3300–treated samples increased from 4.68 to 5.04 and from 4.77 to 5.23, respectively. Exposure of beef samples to the antimicrobial treatments increased the moisture content of samples by 3.3% ± 1.3% (acidified sodium chlorite), 2.9% ± 1.5% (peroxyacetic acid), 5.3% ± 1.3% (sodium metasilicate), 3.5% ± 1.1% (Bromitize Plus), 4.1% ± 1.2% (AFTEC 3000), and 4.0% ± 1.5% (SYNTRx 3300).

Efficacy of decontamination treatments. Analysis of uninoculated beef samples from each of the studies indicated absence (<1 CFU/cm^2) of any naturally occurring rifampin-resistant (on TSA+rif), sorbitol-negative (on mSMAC), and hydrogen sulfide–producing (on XLD agar) microflora. Therefore, colony counts recovered with TSA+rif, mSMAC, and XLD agar from inoculated untreated and treated samples (Tables 3 through 8) were those of the inoculated pathogen. Total bacterial counts of the uninoculated samples ranged from 2.9 ± 0.2 to 4.0 ± 0.6 log CFU/cm^2 (data not shown in tables).

Populations of inoculated *E. coli* O157:H7 on untreated and treated trimmings were enumerated with two selective culture media: TSA+rif, a nonselective medium supplemented with rifampin, and mSMAC, a medium containing several selective ingredients, including potassium tellurite, novobiocin, bile salts, sodium chloride, and crystal violet. Of these two media, TSA+rif provided a less harsh environment for potential recovery of sublethally injured cells after exposure to the decontamination treatments, and therefore higher surviving *E. coli* O157:H7 counts were

TABLE 5. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with 4% sodium metasilicate*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA+rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	Sodium metasilicate	Untreated control	Sodium metasilicate
E. coli O157:H7	3.1 ± 0.0 a A	1.8 ± 0.2 ab B	2.8 ± 0.1 b A	1.4 ± 0.6 b B
Salmonella Newport				
Susceptible	3.2 ± 0.1 a A	1.8 ± 0.3 ab B	3.2 ± 0.1 ab A	1.8 ± 0.3 ab B
MDR-AmpC	3.0 ± 0.1 a A	1.6 ± 0.3 ab B	3.0 ± 0.1 ab A	1.6 ± 0.3 ab B
Salmonella Typhimurium				
Susceptible	3.1 ± 0.1 a A	1.6 ± 0.3 b B	3.1 ± 0.1 ab A	1.6 ± 0.3 ab B
MDR	3.3 ± 0.1 a A	2.0 ± 0.2 a B	3.3 ± 0.1 a A	2.0 ± 0.2 a B
MDR-AmpC	3.1 ± 0.1 a A	1.6 ± 0.5 b B	3.1 ± 0.1 ab A	1.6 ± 0.5 ab B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA+rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

TABLE 6. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with Bromitize Plus (0.0225% active bromine)*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA+rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	Bromitize Plus	Untreated control	Bromitize Plus
E. coli O157:H7	3.1 ± 0.0 a A	2.9 ± 0.1 a B	2.9 ± 0.1 ab A	2.7 ± 0.1 a B
Salmonella Newport				
Susceptible	3.1 ± 0.0 ab A	2.7 ± 0.1 ab B	3.1 ± 0.0 a A	2.7 ± 0.1 a B
MDR-AmpC	3.0 ± 0.0 ab A	2.7 ± 0.1 ab B	3.0 ± 0.0 a A	2.7 ± 0.1 a B
Salmonella Typhimurium				
Susceptible	2.9 ± 0.0 abc A	2.7 ± 0.1 b B	2.9 ± 0.0 ab A	2.7 ± 0.1 a B
MDR	2.9 ± 0.3 bc A	2.5 ± 0.3 bc B	2.9 ± 0.3 ab A	2.5 ± 0.3 ab B
MDR-AmpC	2.8 ± 0.2 c A	2.3 ± 0.2 c B	2.8 ± 0.2 b A	2.3 ± 0.2 b B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA+rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

expected to be obtained on this medium than on mSMAC. However, differences in *E. coli* O157:H7 counts recovered with TSA+rif and mSMAC were numerically small. Mean TSA+rif counts for untreated and treated samples were 0.1 to 0.4 log CFU/cm^2 higher than the corresponding mSMAC counts (Tables 3 through 8). Despite these small differences in log-transformed counts, some significant differences were obtained when TSA+rif and mSMAC counts were separately compared with the counts of the tested *Salmonella* inocula (recovered on XLD agar). For this reason, results of both sets of comparisons (i.e., TSA+rif counts compared with XLD agar counts and mSMAC counts compared with XLD agar counts) are presented (Tables 3 through 8).

Reductions within each decontamination treatment were similar among all inocula (Tables 3 through 8). Overall, depending on chemical treatment, initial counts of *E. coli* O157:H7 (3.1 [TSA+rif] and 2.7 to 3.0 [mSMAC] log CFU/cm^2) were reduced ($P < 0.05$) by 0.2 to 1.4 log CFU/cm^2 (Tables 3 through 8). Similarly, initial counts of the tested *Salmonella* inocula (2.8 to 3.3 log CFU/cm^2) were reduced ($P < 0.05$) by 0.4 to 1.4 (*Salmonella* Newport, antibiotic susceptible), 0.3 to 1.4 (*Salmonella* Newport, MDR-AmpC), 0.2 to 1.5 (*Salmonella* Typhimurium, antibiotic susceptible), 0.4 to 1.3 (*Salmonella* Typhimurium, MDR), and 0.4 to 1.5 (*Salmonella* Typhimurium, MDR-AmpC) log CFU/cm^2, depending on the antimicrobial

TABLE 7. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with AFTEC 3000 (pH 1.2)*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA+rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	AFTEC 3000	Untreated control	AFTEC 3000
E. coli O157:H7	3.1 ± 0.1 a A	2.8 ± 0.1 a B	3.0 ± 0.1 b A	2.6 ± 0.1 a B
Salmonella Newport				
Susceptible	3.2 ± 0.0 a A	2.6 ± 0.1 b B	3.2 ± 0.0 a A	2.6 ± 0.1 a B
MDR-AmpC	3.1 ± 0.1 ab A	2.5 ± 0.1 bc B	3.1 ± 0.1 ab A	2.5 ± 0.1 ab B
Salmonella Typhimurium				
Susceptible	3.0 ± 0.1 ab A	2.5 ± 0.2 bc B	3.0 ± 0.1 ab A	2.5 ± 0.2 ab B
MDR	3.1 ± 0.1 a A	2.4 ± 0.2 cd B	3.1 ± 0.1 ab A	2.4 ± 0.2 bc B
MDR-AmpC	2.9 ± 0.0 b A	2.3 ± 0.2 d B	2.9 ± 0.0 b A	2.3 ± 0.2 c B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA+rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

TABLE 8. *Populations of rifampin-resistant* E. coli *O157:H7 and antibiotic-susceptible and multidrug-resistant* Salmonella *Newport and* Salmonella *Typhimurium on beef trimmings before (untreated control) and after decontamination with SYNTRx 3300 (pH 1.0)*[a]

	Bacterial counts recovered (log CFU/cm^2)[b]			
	TSA+rif (*E. coli* O157:H7) and XLD (*Salmonella*)		mSMAC (*E. coli* O157:H7) and XLD (*Salmonella*)	
Inoculum	Untreated control	SYNTRx 3300	Untreated control	SYNTRx 3300
E. coli O157:H7	3.1 ± 0.1 a A	2.8 ± 0.1 a B	3.0 ± 0.1 ab A	2.5 ± 0.2 b B
Salmonella Newport				
Susceptible	3.2 ± 0.0 a A	2.8 ± 0.1 ab B	3.2 ± 0.0 a A	2.8 ± 0.1 a B
MDR-AmpC	3.1 ± 0.1 ab A	2.6 ± 0.1 c B	3.1 ± 0.1 ab A	2.6 ± 0.1 ab B
Salmonella Typhimurium				
Susceptible	3.0 ± 0.1 ab A	2.6 ± 0.1 bc B	3.0 ± 0.1 ab A	2.6 ± 0.1 ab B
MDR	3.1 ± 0.1 ab A	2.6 ± 0.2 c B	3.1 ± 0.1 ab A	2.6 ± 0.2 ab B
MDR-AmpC	2.9 ± 0.0 b A	2.5 ± 0.1 c B	2.9 ± 0.0 b A	2.5 ± 0.1 b B

[a] Rifampin-resistant *E. coli* O157:H7 was recovered with TSA+rif (tryptic soy agar supplemented with 100 μg/ml rifampin) or mSMAC (modified sorbitol MacConkey agar). *Salmonella* Newport and *Salmonella* Typhimurium inocula were recovered with XLD (xylose lysine deoxycholate) agar.

[b] Values are mean ± standard deviation, $n = 6$. Within a column, means with a common lowercase letter are not significantly different ($P \geq 0.05$). Within a row and within each culture medium, means with a common uppercase letter are not significantly different ($P \geq 0.05$).

treatment (Tables 3 through 8). Ranges of pathogen reductions obtained for each of the chemical treatments were 0.4 to 0.6 (acidified sodium chlorite), 0.5 to 0.7 (peroxyacetic acid), 1.3 to 1.5 (sodium metasilicate), 0.2 to 0.5 (Bromitize Plus), 0.3 to 0.7 (AFTEC 3000), and 0.3 to 0.5 (SYNTRx 3300) log CFU/cm^2 for all tested inocula (Tables 3 through 8). Because of the experimental design and execution of the study, it was not possible to statistically compare results among chemical treatments; results could only be compared between the inocula within each chemical treatment. Total bacterial counts of inoculated untreated samples ranged from 3.3 to 4.2 log CFU/cm^2, and reductions obtained after decontamination with the tested antimicrobials were generally similar or at most 0.5 log CFU/cm^2 lower than those observed for the individual pathogen inocula (data not shown).

Regardless of antimicrobial treatment, counts of surviving *E. coli* O157:H7 recovered with TSA+rif or mSMAC from decontaminated samples were generally similar ($P \geq 0.05$) or higher ($P < 0.05$) than those of the multidrug-resistant and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium inocula (Tables 3 through 8). In a few instances, counts of surviving *E. coli* O157:H7 on treated trimmings were lower ($P < 0.05$) than those of some of the *Salmonella* inocula (Tables 3 through 5 and 8); however, in all of these cases, the difference between the *E. coli* O157:H7 counts and the specific *Salmonella* inoculum counts was 0.2 to 0.6 log CFU/cm^2. The small variation observed among counts for samples within and between the pathogen inoculum treatments (Tables 3 through 8) is one likely reason that differences in mean counts as low as 0.2 log CFU/cm^2 were significant ($P < 0.05$). Another possible reason could be the small differences in the initial levels of the pathogen inocula on untreated control samples. For example, in the study with sodium metasilicate, *E. coli* O157:H7 counts recovered with mSMAC were 0.5 log CFU/cm^2 lower ($P < 0.05$) than those of MDR *Salmonella* Typhimurium on untreated trimmings, and a similar difference ($P < 0.05$) of 0.6 log CFU/cm^2 was observed for the counts of these inocula on the treated samples (Table 5).

Comparison of survivors of the tested *Salmonella* Newport and *Salmonella* Typhimurium inocula on treated trimmings indicated that in general serotype and antibiotic resistance phenotype did not ($P \geq 0.05$) influence the efficacy of the decontamination treatments (Tables 3 through 8). As noted previously with the *E. coli* O157:H7 counts, in only a few cases were significant differences ($P < 0.05$) obtained within or between the *Salmonella* serotypes and/or antibiotic resistance phenotypes, but the numerical differences in counts in these instances were only 0.2 to 0.4 log CFU/cm^2. Such small log-unit differences are not considered biologically significant *(16)*.

Other studies *(12, 17, 18)* have been conducted on the efficacy of some of the antimicrobials tested here (i.e., acidified sodium chlorite, peroxyacetic acid, and sodium metasilicate) against *E. coli* O157:H7 or *Salmonella* on beef trimmings; however, unlike in the present study, in those studies the antimicrobial effects of the treatments were not compared among different inocula. In one study *(2)* with a design similar to that of the present study, various acid (lactic acid, acetic acid, and FreshFX) and nonacid (hot water, electrolyzed water, and ozone) treatments were evaluated against *E. coli* O157:H7 and antibiotic-susceptible *Salmonella* Newport and *Salmonella* Typhimurium on beef tissue samples; these treatments were equally or more effective against multidrug-resistant phenotypes of the *Salmonella* serotypes.

In conclusion, the results of this study indicate that the chemical decontaminating interventions evaluated under the conditions examined should be of at least similar efficacy on beef trimmings contaminated with *Salmonella* or *E. coli* O157:H7. Multidrug-resistant strains of *Salmonella* Newport and *Salmonella* Typhimurium should be as sensitive to the decontamination treatments as are antibiotic-susceptible strains of these serotypes.

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