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Relative response of populations of *Escherichia coli* and *Salmonella enterica* to exposure to thermal, alkaline and acidic treatments

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ABSTRACT

We evaluated the relative response of generic *Escherichia coli* (GEC), Shiga toxin-producing *E. coli* (STEC) and *Salmonella enterica* to heat, alkaline or acid treatments. GEC included strains from carcasses (n = 24) and trim (n = 25) at a small beef plant where no decontamination interventions are used and at a large plant where multiple decontamination interventions are used (carcass n = 25 and trim n = 25). STEC strains belonging to nine serogroups, included isolates from cattle (n = 53), beef (n = 16) and humans (n = 44). *S. enterica* strains belonging to 29 serotypes, included isolates from humans (n = 30), poultry (n = 26), pork (n = 10) and beef (n = 33). Strains were grown in Brain Heart Infusion (BHI) broth and subjected to the following treatments: 60 °C for 2 min, 5% lactic acid (pH 2.9) for 1 h at 4 °C, or NaOH (pH 11.0) for 2 h at 4 °C. Median log reductions of the GEC populations after heat, alkaline and acid treatment ranged from 2.3 to 3.8, 0.7 to 2.2 and 0.7 to 1.2 log CFU/mL, respectively. No statistically significant difference in reductions was observed between carcass GEC or trim GEC from the large or small plant, except for a greater reduction in trim GEC from the small plant. Median reductions of the STEC populations ranged from 3.3 to 3.5, 0.0 to 0.6, and 0.3 to 0.5 log CFU/mL after heat, alkaline and acid treatment, respectively. The median reductions were not dependent upon isolation source, except between STEC cattle and human isolates after alkaline treatment, where the reduction of the former was higher by 0.6 log unit. For the *Salmonella* populations, median log reductions ranged from 3.5 to 4.0, 1.7 to 2.4 and 3.7 to 4.1 log CFU/mL after heat, alkaline and acid treatment, respectively. The reductions were not isolation source related. The median log reductions were in the order GEC < STEC < *Salmonella* after heat treatment and STEC < GEC < *Salmonella* after alkaline or acid treatment. Overall, the relative response of GEC, STEC and *Salmonella* in the model system suggests that exposure to heat or pH-based decontamination interventions in meat plants is not associated with increased resistance among *E. coli* strains in these environments, and total *E. coli* counts on beef can be indicative of treatment efficacy for the control of *Salmonella* by heat, lactic acid and alkaline treatment and for the control of STEC subjected to heat.

1. Introduction

It has been estimated that every year there are 4 million cases of domestically acquired foodborne illness in Canada (Thomas et al., 2013), resulting in 3200 to 4800 hospitalizations and 75 to 139 deaths (Thomas et al., 2015). Enteric pathogens such as *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC, also known as verotoxin-producing *E. coli*) are a major cause of foodborne disease outbreaks. Infections with STEC most commonly manifest as uncomplicated diarrhea, which may develop into a self-limiting bloody diarrhea, but 5–10% of people may develop life threatening hemolytic-uremic syndrome (Karpman and Ståhl, 2014). Non-typhoidal salmonellosis is usually characterized by a self-limiting enterocolitis with diarrhea in immunocompetent individuals, but may cause more severe illness or long term sequelae in sensitive groups and immunocompromised individuals (Majowicz et al., 2010). Outbreaks of *Salmonella* and STEC are often associated with consumption of undercooked meat (Bélanger et al., 2015; Thomas et al., 2013). Despite the efforts made to maintain good hygiene practices during meat production, complete prevention of bacterial contamination of carcasses is not yet attainable. This is a consequence of the inevitable transfer of bacteria from the hide of the animal and the meat plant environment to bacteria-free muscle tissue, as surfaces are exposed during slaughter and carcass breaking (Bacon et al., 2000; Bell, 1997; Nottingham, 1982). To control the contamination of beef with enteric pathogens, many beef processing plants in North America have implemented various interventions to reduce

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bacterial loads through decontamination, particularly during the carcass dressing process, as part of their Hazard Analysis Critical Control Points (HACCP) programs (Gill, 2009). Commonly used decontamination treatments at commercial plants include washing hide-on carcasses with a solution of 1.5% NaOH (Bosilevac et al., 2005), pasteurizing carcasses with hot water (85 °C) or steam (> 90 °C), and spraying carcasses with organic acid solutions such as lactic, acetic or peroxyacetic acid at various concentrations. Hot water and organic acid treatments have also been used in poultry and pork processing facilities. Application of these treatments and their microbiological effects in commercial settings has been reviewed (Gill, 2009; Loretz et al., 2010, 2011b).

STEC and *Salmonella* are often found on meat at low prevalence and at very low numbers, making it impractical to obtain enough samples to reliably estimate their numbers in meat for the purpose of assessing the performance of the production process in terms of microbial hygiene (Brown et al., 2000). Generic *E. coli* has long been regarded as an indicator organism for the possible contamination of meat with enteric pathogens as they share the same natural habitat, and generic *E. coli* is present in larger numbers than the pathogens of concern. Consequently, numerous studies have used total *E. coli* counts to assess the efficacy of decontamination treatments in beef, pork and poultry plants (Gill, 2009; Loretz et al., 2010, 2011a, 2011b). In addition, regulatory bodies across the world have identified total *E. coli* counts as a potential indictor of process hygiene (CFIA, 2014; EFSA, 2010, 2011; FSIS, 2001). Previous studies have compared the efficacy of antimicrobial treatments on beef against surrogate *E. coli* strains and enteric pathogens (Kalchayanand et al., 2015; Kalchayanand et al., 2012; Keeling et al., 2009; Niebuhr et al., 2008), but published accounts on whether the response of naturally occurring generic *E. coli* to interventions, as measured by total *E. coli* counts, is indicative of the response of STEC and *Salmonella* are scarce. When strains of the same bacterial species are treated under identical conditions, the response may vary significantly among the strains (Whiting and Golden, 2002). This is could be due to differences in the genes and gene variants carried by individual strains or differences in the regulation of gene expression. Both the total genome size and the contents of the accessory genome can vary dramatically between strains of the same bacterial species (Hayashi et al., 2001). Furthermore, some researchers have raised concerns that decontamination treatments applied at meat packing plants may select for resistant populations of *E. coli* and lead to reduced susceptibility and even cross resistance to other decontamination treatments (Dlusskaya et al., 2011; Soumet et al., 2012). The objectives of this study were to determine 1) whether the range and distribution of the response among a population of generic *E. coli* is indicative of the response of STEC and *Salmonella* to interventions commonly used in meat plants; 2) whether the application of decontamination treatments in meat plants results in increased resistance to those treatments among the population of generic *E. coli*; 3) whether the relative response of STEC and *Salmonella* to decontamination treatments is isolation source or serotype dependent. To do so, we used a population approach to examine the reduction following thermal, alkaline or acid treatments of generic *E. coli* from meat that had not or had been exposed to decontamination interventions, and of STEC and *Salmonella* from various sources. The bacteria were exposed to thermal, alkaline and acid treatments, in an in vitro model system to permit screening of large populations of strains while simulating the interventions commonly used in meat processing plants.

2. Materials and methods

2.1. Bacterial strains

Experiments were conducted upon a total of 99 strains of generic *E. coli*, 113 strains of STEC and 99 strains of *Salmonella enterica*. All generic *E. coli* strains were from the culture collection of the Lacombe Research and Development Centre of Agriculture and Agri-Food Canada. The generic *E. coli* strains were isolated from beef carcasses or trim at a small and a large plant in previous studies (Visvalingam et al., 2016; Yang et al., 2015a; Yang et al., 2017a; Yang et al., 2015b). The generic *E. coli* isolates were grouped as carcass small plant (CS n = 24), carcass large plant (CL n = 25), trim small plant (TS n = 25) and trim large plant (TL n = 25). During the period of strain isolation, the large plant employed the following decontamination interventions during the carcass dressing process: hide-on carcass wash with 1.5% NaOH, spraying carcasses with 5% lactic acid, and pasteurization of carcasses with steam at > 90 °C (Yang et al., 2012). *E. coli* strains from carcasses at the large plant were of genome types, as determined by MLVA, that were found on both carcass sides at the end of the dressing line, i.e. carcasses that had been exposed to all three decontamination interventions, and hides before hide-on wash, representing a presumed resistant population (Yang et al., 2015a). The small plant did not use any decontamination interventions, other than washing carcasses with cold water, and so represent a population that has not been exposed to the same decontamination processes (Liu et al., 2016). The STEC group included 50 O157 strains, including 40 strains originally isolated from Canadian feedlot cattle (Aslam et al., 2010), 10 strains each of serogroups O26, O103, O121 and O145, and 11 O111, 8 O45, 2 O113, 1 O91, and 1 O104. STEC strains O26-2, O26-3, O45-3, O45-4, O103-2, O103-3, O111-3, O111-4, O121-3, O121-4, O145-2, and O145-3, were originally isolated from cattle and were kindly supplied by Dr. Rong Wang of USDA-ARS (Clay Center, NE). The remaining STEC strains and all *Salmonella* strains, a mix of 29 serotypes, were from the Health Canada, Bureau of Microbial Hazards culture collection. Overall, the STEC population included 53, 16 and 44 strains isolated from cattle, beef and human, respectively. The *Salmonella* population included 30, 26, 10, and 33 strains isolated from human, poultry, pork and beef, respectively. All bacterial strains were stored as glycerol stocks or on CryoStor beads at −80 °C.

2.2. Development of experimental system

The aim of the experimental system was to collect data from large populations of bacterial strains on the relative response to decontamination treatments used in meat processing. Cells were suspended in Brain Heart Infusion (BHI), an animal tissue based broth media, to ensure that the cells would have access to the substrates that might be used for active stress resistance on meat surfaces and organic molecules which may potentially protect from or potentiate the treatments (Kalchayanand et al., 2016). The cells were maintained prior to treatment at 4 °C for 48 h, to model exposure to stress under refrigerated conditions and to equilibrate the cells to a consistent physiological state. The treatment conditions, 60 °C for 2 min, 5% lactic acid for 1 h and pH 11 NaOH for 2 h, were selected following preliminary experiments with 10 generic *E. coli* strains. The treatment conditions used were selected for producing surviving cell numbers quantifiable by direct plating.

2.3. Bacterial suspension preparation

The bacterial strains were each streaked onto BHI agar and incubated at 35 °C for 24 h. Single colonies were inoculated into 10 mL of BHI broth which was incubated at 35 °C for 24 h. The overnight cultures were transferred to 15 mL Falcon tubes and harvested by centrifugation (Eppendorf Centrifuge 5430, Hamburg, Germany) at 6000 rpm (4226 rcf) for 10 min. The supernatant was discarded and the cell pellet was resuspended in 10 mL of phosphate buffered saline (PBS; pH 7.00). The bacterial suspensions were centrifuged again at 6000 rpm for 10 min. The pelleted cells were resuspended in 10 mL of 4 °C fresh BHI broth. The BHI cell suspension was adjusted by dilution to an optical density (OD) of 0.100 ± 0.005 (approximately 7.5 log CFU/mL) as measured by a spectrophotometer at a wavelength of 600 nm. The OD adjusted cell suspensions were stored at 4 °C for a minimum of 48 h before

Table 1
Statistics for reduction (log CFU/mL) of generic *Escherichia coli* (GEC), *Salmonella* and Shiga toxin-producing *E. coli* (STEC) after heat, alkaline and acid treatments[a].

Bacteria	n	Range/IQR[b]			Median[c]			CV[d]		
		Thermal	Alkaline	Acid	Thermal	Alkaline	Acid	Thermal	Alkaline	Acid
GEC	99	0.8– > 5/1.1	0–3.3/1.5	0–3.5/0.8	2.8A	0.9A	0.9A	30.2	77.4	64.5
Salmonella	99	2.1– > 5/0.8	0– > 5/1.8	0.5– > 5/1.9	3.7B	2.3B	4.0B	16.9	60.4	31.8
STEC	113	1.7– > 5/0.9	0–2.5/0.8	0–1.8/0.5	3.3C	0.2C	0.5C	18.4	129.6	80.0

[a] The conditions were incubation at 60 °C for 2 min, at pH 2.9 for 1 h at 4 °C, and at pH 11.0 for 2 h at 4 °C.
[b] IQR, interquartile.
[c] Median values that do not share a common letter are significantly different ($p < 0.05$) within each treatment group.
[d] CV, coefficient of variation.

experimental use. On the day of the experiment, the OD adjusted cell suspensions were serially diluted in PBS to 10^{-5} and 100 µL of the highest dilution was spiral plated (EddyJet 2, IUL Instruments, Barcelona, Spain) onto Tryptic Soy agar (TSA) which was incubated at 35 °C for 48 h.

2.4. Thermal treatment

Relative response of the strains to thermal treatment was determined by exposure to 60 °C for 2 min. Briefly, the OD adjusted cell suspensions were taken out of the fridge and vortexed to ensure a homogenous mixture of cells throughout each tube. An aliquot of 100 µL of the cell suspension was added to 9.9 mL of BHI broth pre-warmed to 60 °C in a water bath operated at the same temperature. Five seconds prior to the end of the 2 min thermal treatment the tube was vortexed, to mix the cells, and returned to the water bath, then at the completion of the 2 min exposure, 500 µL of the cell suspension was rapidly cooled by immediate addition to a test tube containing 4.5 mL of PBS pre-chilled in an ice water bath. The chilled suspension was serially diluted in PBS to 10^{-3} and 100 µL aliquots of the thermally treated cell suspension and each dilution were spread plated in duplicate onto TSA plates which were incubated at 35 °C for 48 h.

2.5. Acid and alkaline treatment

Acid and alkaline treatment were carried out in BHI supplemented with lactic acid or NaOH, respectively. For the acid BHI solution, 90% lactic acid and distilled water were added to double strength (2×) BHI broth to create a 5% lactic acid BHI solution with a pH of 2.9. For the alkaline BHI solution, 5 N NaOH and distilled water were added to 2× BHI broth to create a pH 11.0 BHI solution. The acid or alkaline BHI solution was sterilised by filtration (0.02 µm GV PVDF Membrane Stericup, EMD Millipore Stericup, Barcelona, Spain) and dispensed into 15 mL Falcon tubes, which were stored at 4 °C until use.

Aliquots of 100 µL of the OD adjusted cell suspension of each strain were added to the acid or alkaline BHI solutions. After vortexing briefly, the tubes were incubated at 4 °C for 1 h (lactate) and 2 h (NaOH), respectively. At the end of the treatment period, the tubes were vortexed again to mix and serial dilutions in PBS up to 10^{-2} were made. Undiluted treated cells were spread plated, 200 µL, onto duplicate TSA. Aliquots of 50 µL of each PBS dilution were spiral plated in duplicate onto TSA. The plates were incubated at 35 °C for 48 h.

2.6. Colony enumeration and data analysis

The data presented are means from duplicate biological replicates. Each experiment was repeated twice for each strain, with an independent culture on different days. After incubation, the colony forming units (CFU) on the agar plates were enumerated and log CFU/mL was calculated. The mean log CFU/mL for the duplicate plates of control and treated cell suspensions was determined and log reduction calculated ($\log \text{CFU/mL}_{\text{untreated control}} - \log \text{CFU/mL}_{\text{treatment}}$).

The log reductions were grouped into sets according to treatment (thermal, alkaline, and acid) and source of isolation (CL, CS, TL, and TS for generic *E. coli*; human, poultry, pork and beef for *Salmonella*; and cattle, beef and human for STEC). For STEC, log reductions were also grouped by O157 and non-O157, and within the non-O157 group by O-antigen. For *Salmonella*, log reductions were grouped according to serotypes for Enteritidis (n = 5), Heidelberg (n = 7) and Typhimurium (n = 11), the three *Salmonella* serotypes most commonly clinically isolated in Canada. Some of those data sets were not normally distributed, as determined by Shapiro-Wilk test (SAS 9.4, SAS Institute, Cary, NC). As such, for all data sets median values were compared using the Dunn test, a nonparametric multiple comparison procedure in SAS. A significance level of 0.05 was used for all statistical analysis.

3. Results

3.1. Relative response of generic E. coli, STEC and Salmonella to thermal, alkaline and acid treatment

The relative resistance to thermal, lactic acid or alkaline treatment, as measured by log reduction, was observed to vary by up to five orders of magnitude between individual strains of the generic *E. coli* (n = 99), STEC (n = 113) and *Salmonella* (n = 99) (Table 1). Variability, as determined by interquartile range (IQR) and coefficient of variation (CV), was dependent on treatment as well as grouping of the organisms. Thermal reductions for individual strains ranged from 0.8 to > 5 log among generic *E. coli*, from 1.7 to > 5 log among STEC, and from 2.1 to > 5 log among *Salmonella*. The range of response of individual *Salmonella* strains to lactic acid or alkaline treatment was also high, with maximum log reductions of > 5 and minimum log reductions of 0 (alkaline) or 0.5 (lactic acid). A narrower range of resistance was observed in response to pH stress by generic *E. coli*, with individual strains of generic *E. coli* being reduced by 0 to 3.5 log following lactic acid treatment and 0 to 3.3 log in response to alkaline treatment. The range of response of STEC strains to pH stress was the narrowest, with reductions from lactic acid treatment ranging from 0 to 1.8 log and 0 to 2.5 log in response to alkaline treatment. The median log reductions of the generic *E. coli*, *Salmonella* and STEC after thermal, acid or alkaline treatment were significantly different ($p < 0.05$). For thermal treatment, the median log reduction was in the order generic *E. coli* < STEC < *Salmonella*. For alkaline and acid treatment, the median log reduction was in the order STEC < generic *E. coli* < *Salmonella*.

3.2. Response of generic E. coli to thermal, acid and alkaline treatment

The log reductions of *E. coli* strains resulting from each of the three treatments varied greatly within each population (Fig. 1). Reductions after heat treatment of the *E. coli* populations from CS, CL, TS, and TL ranged from 2.3 to 4.1, 0.8 to 3.8, 1.9 to 5.0, and 0.9 to 4.6 log CFU/mL, respectively. The respective median reductions were 2.9, 2.7, 3.8, and 2.3 log CFU/mL. The median log reduction of the TS population was significantly ($p < 0.05$) higher than that of the TL, CL, but not CS

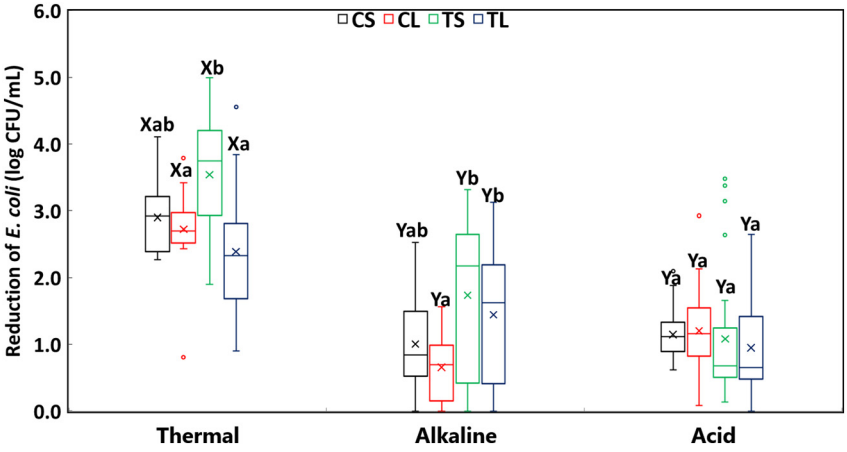

Fig. 1. Reduction of *Escherichia coli* isolated from carcasses at a small plant (CS), carcasses at a large plant (CL), trim at a small plant (TS), and trim at a large plant (TL) after thermal (60 °C for 2 min), alkaline (pH 11 for 2 h) and acid (pH 2.9 for 1 h) treatments. Small letters and capital letters denote comparisons of median reductions within a treatment and across treatments, respectively. Reductions in each category that do not share a common letter are significantly different ($p < 0.05$). Boxes indicate data within the first and third quartile (interquartile range, IQR), central horizontal lines indicate medians, x marks indicate means, and whiskers indicate the lowest data point within 1.5 IQR of the first quartile, and the highest data point within 1.5 IQR of the third quartile. Open circles indicate data points above or below the 1.5 IQR of the first or third quartile.

($p > 0.05$) populations. There were no significant differences in median log reductions following thermal treatment between any of the other generic *E. coli* populations ($p > 0.05$).

Incubation with lactate at pH 2.9 for 1 h resulted in reductions of the *E. coli* populations from CS, CL, TS, and TL by 0.6 to 2.1, 0.1 to 2.9, 0.2 to 3.5, and 0.0 to 2.7 log CFU/mL, respectively (Fig. 1). The respective median values were 1.1, 1.2, 0.7 and 0.7 log CFU/mL. The median reductions following lactate treatment did not differ significantly ($p > 0.05$).

Reductions after alkaline treatment of the *E. coli* populations from CS, CL, TS and TL ranged from 0.0 to 2.5, 0.0 to 1.6, 0.0 to 3.3 and 0.0 to 3.1 log CFU/mL, respectively. The respective median reductions were 0.8, 0.7, 2.2 and 1.6 log CFU/mL (Fig. 1). There was no significant difference ($p > 0.05$) in median value between the two *E. coli* populations from carcasses or the two populations from trim. The two trim populations had significantly higher ($p < 0.05$) median reductions following alkaline treatment than the carcass population from the large plant, but they did not differ significantly ($p > 0.05$) from the carcass population from the small plant.

The median reductions of *E. coli* after thermal treatment were significantly higher ($p < 0.05$) than those after alkaline or acid treatment, irrespective of the source of isolation (Fig. 1). No significant difference ($p > 0.05$) was noted between alkaline and acid treatment for any of the four populations.

3.3. Response of STEC to thermal, acid and alkaline treatment

Reductions after thermal treatment of the STEC populations from cattle, beef and human ranged from 2.3 to 4.8, 2.2 to 4.3, and 1.7 to 5.0 log CFU/mL, respectively (Fig. 2). The respective median values were 3.4, 3.5 and 3.3 log CFU/mL. These median values were not significantly different ($p > 0.05$).

The reductions after acid treatment of STEC from cattle, beef and human ranged from 0.0 to 1.8, 0.0 to 1.1 and 0.0 to 1.5 log CFU/mL, respectively. The corresponding median values were 0.5, 0.4 and 0.3 log CFU/mL, respectively. As with thermal treatment, no significant difference ($p > 0.05$) in log median reduction after acid treatment between STEC from the three sources was observed.

Reductions after alkaline treatment ranged from 0.0 to 2.3, 0.0 to 2.5 and 0.0 to 1.9 log CFU/mL for STEC from cattle, beef and human, respectively. The respective median values were 0.6, 0.0 and 0.1 log CFU/mL. The median reduction of STEC from human was significantly lower ($p < 0.05$) than that of STEC from cattle. There was no significant difference ($p > 0.05$) in the median log reduction between STEC from cattle and beef, or beef and human.

As with generic *E. coli*, the median reduction of STEC after thermal treatment was significantly higher ($p < 0.05$) than reductions following alkaline or acid treatment, irrespective of the source of isolation

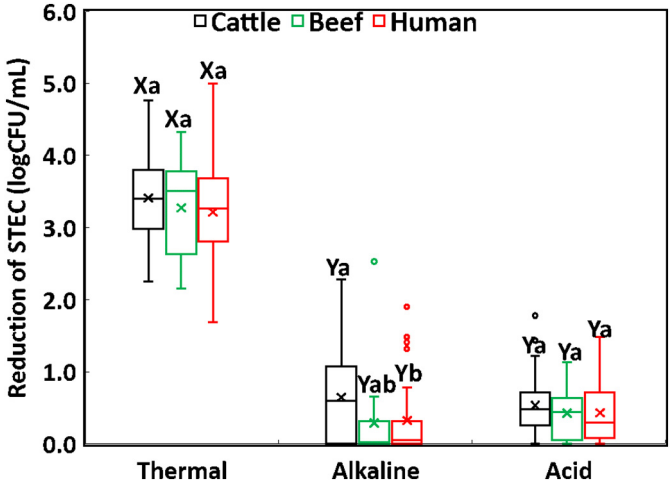

Fig. 2. Reduction of Shiga toxin-producing *Escherichia coli* (STEC) isolated from cattle, beef, and human after thermal (60 °C for 2 min), alkaline (pH 11 for 2 h) and acid (pH 2.9 for 1 h) treatments. Small letters and capital letters denote comparisons of median reductions within a treatment and across a treatment, respectively. Reductions that do not share a common letter are significantly different ($p < 0.05$). Boxes indicate data within the first and third quartile (interquartile range, IQR), central horizontal lines indicate medians, x marks indicate means, and whiskers indicate the lowest data point within 1.5 IQR of the first quartile, and the highest data point within 1.5 IQR of the third quartile. Open circles indicate data points above or below the 1.5 IQR of the first or third quartile.

(Fig. 2). No significant difference ($p > 0.05$) was noted between alkaline and acid treatment for any of the STEC populations.

3.4. Response of Salmonella to thermal, acid and alkaline treatment

Reductions of *Salmonella* from human, poultry, pork and beef ranged from 2.1 to 5.0, 3.0 to 5.0, 2.6 to 4.4, and 2.7 to 5.0 log CFU/mL after thermal treatment; 0.3 to 5.0, 0.1 to 4.9, 0.0 to 4.6, and 0.0 to 4.9 after alkaline treatment; 1.1 to 5.0, 0.9 to 5.0, 0.9 to 5.0, and 0.5 to 5.0 log CFU/mL after acid treatment (Fig. 3). The respective median reductions were 4.0, 3.6, 3.5, and 3.6 log CFU/mL after thermal treatment, 2.4, 2.5, 1.9, and 1.7 log CFU/mL after alkaline treatment, and 4.1, 3.8, 3.7, and 4.1 log CFU/mL after acid treatment. The four *Salmonella* populations did not differ in their median log reductions, irrespective of the treatment type.

For all four *Salmonella* populations, median reductions after acid treatment were significantly higher ($p < 0.05$) than those after alkaline treatment, but were not significantly different ($p > 0.05$) from those after thermal treatment (Fig. 3).

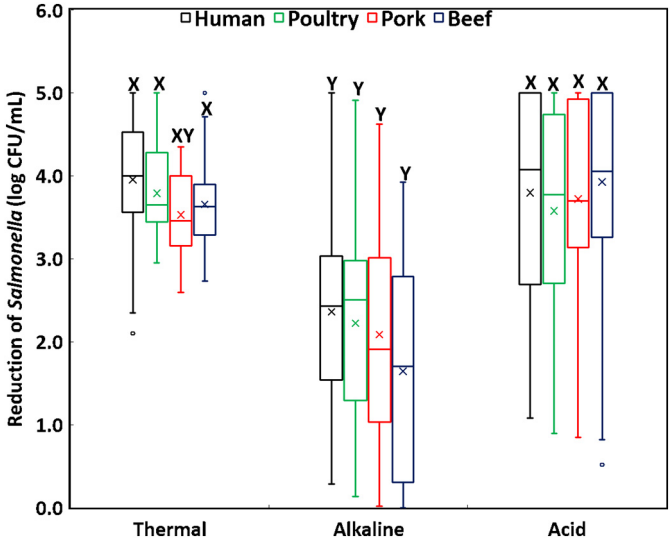

Fig. 3. Reduction of *Salmonella enterica* isolated from human, poultry, pork and beef after thermal (60 °C for 2 min), alkaline (pH 11 for 2 h) and acid (pH 2.9 for 1 h) treatments. Letters denote comparisons of median reductions of *Salmonella* from a common isolation source to different treatments. Reductions that do not share a common letter are significantly different ($p < 0.05$). Boxes indicate data within the first and third quartile (interquartile range, IQR), central horizontal lines indicate medians, x marks indicate means, and whiskers indicate the lowest data point within 1.5 IQR of the first quartile, and the highest data point within 1.5 IQR of the third quartile. Open circles indicate data points above or below the 1.5 IQR of the first or third quartile.

3.5. Correlation between thermal, acid and alkaline resistance

Log reductions after thermal, alkaline and acid treatments of all strains of generic *E. coli*, STEC or *Salmonella* were replotted using scatter plot to discern potential correlations between reductions resulting from the treatments (Fig. S1). Pearson correlation coefficient (r) values between reductions of *E. coli* after thermal and alkaline, thermal and acid, and acid and alkaline treatments were 0.15, 0.10 and 0.18, respectively. None of these correlations were significant ($p > 0.05$). Pearson correlation coefficient values between reductions of STEC after thermal and alkaline, thermal and acid, and acid and alkaline treatments were 0.28, 0.45 and 0.39, respectively. These correlations were significant ($p < 0.05$). Pearson correlation coefficient values between reductions of *Salmonella* after thermal and alkaline, thermal and acid, and acid and alkaline treatments were 0.06, 0.01 and 0.33, respectively. The correlation between alkaline and acid treatment was significant ($p < 0.05$).

3.6. Relative response of major serogroups of STEC and Salmonella to the three treatments

Median log reductions of STEC O157 (n = 50) and STEC non-O157 (n = 63) were 3.5 and 3.3 log CFU/mL after heat treatment, and 0.5 and 0.3 log CFU/mL after acid treatment, respectively. These two pairs of median values were not significantly different ($p > 0.05$). The median log reductions after alkaline treatment of STEC O157 and STEC non-O157 were 0.7 and 0.0 log CFU/mL, respectively, and they were significantly different ($p < 0.05$). The six major non-O157 STEC serogroups O26 (n = 10), O45 (n = 8), O103 (n = 10), O111 (n = 11), O121 (n = 10) and O145 (n = 10) were compared for their response to thermal, acid and alkaline treatment (Fig. 4). The median reductions after thermal treatment of O145 and O111 were 2.4 and 3.8 log CFU/mL, respectively, and they were significantly different ($p < 0.05$). Median log reductions of the serogroup O111 after acid and alkaline treatment were 0.6 and 1.0 log CFU/mL and were higher than the corresponding median log reductions of the serogroup O145 (0.0 log CFU/mL), and O26, O121 and O145, respectively. The median reduction for the latter three serotypes was 0.0 log CFU/mL. No other significant differences between the serotypes were found.

Median log reductions of *Salmonella* Enteritidis (n = 5), Heidelberg (n = 7) and Typhimurium (n = 11) were 3.5, 3.7, 3.6 log CFU/mL after heat treatment, 2.6, 2.7, 1.9 log CFU/mL after alkaline treatment, and 3.5, 4.1 and 4.6 log CFU/mL after acid treatment, respectively. The reductions between the three serotypes were not significantly different ($p > 0.05$), irrespective of the treatment type.

4. Discussion

Phenotypic variability in response to stress can be expected to be more prominent at relatively mild conditions and the rate of reduction can be expected to converge as treatments increase in intensity. This phenomenon is of particular significance for both physical and chemical decontamination interventions for carcasses, as a wide range of treatment intensities on meat surface can be expected due to physical and chemical characteristics of meat. In this study, we treated a large number of strains of generic *E. coli*, STEC and *Salmonella* under relatively mild conditions (60 °C for 2 min, 5% lactic acid pH 2.9 for 1 h or pH 11 for 2 h) to allow quantification of the population response under conditions relevant to decontamination interventions in meat plant settings.

E. coli, in general, is not particularly heat resistant, with reported D_{60}-values being mostly < 2 min (Stringer et al., 2000; van Asselt and Zwietering, 2006). However, an extremely heat resistant *E. coli* strain from a beef plant has been reported with a D_{60}-value of 71 min and the authors suggested that the extreme heat resistance of *E. coli* is a result of selection for this characteristic in response to pasteurization of beef carcasses (Dlusskaya et al., 2011). *E. coli* heat resistance can be attributed to the presence of small heat shock proteins (Han et al., 2008), accumulation of compatible solutes (Pleitner et al., 2012), and proteins involved in cell envelope maintenance and osmotic stress regulations (Mercer et al., 2015). In the present study, the median log reductions after incubation at 60 °C for 2 min of *E. coli* strains isolated from carcasses at a plant where carcass pasteurization with steam is routinely used and from carcasses at a plant where no decontamination interventions for carcasses are used were not significantly different. The heat resistance of all the *E. coli* strains tested was in line with what would be expected for most *E. coli*. This suggests that carcass pasteurization does not impose a selection pressure for extreme heat resistance in *E. coli*. Interestingly, the median log reduction of *E. coli* from trim from the plant where no decontamination interventions are used was 1.4 log units higher than that of *E. coli* from trim from the plant where multiple decontamination interventions are used. Even so, *E. coli* strains with extreme heat resistance, defined as D_{60} value being ≥ 10 min, were not observed. Some recent studies in which the source of *E. coli* on beef trimmings were identified by genotyping have demonstrated that the surfaces of beef fabrication equipment could be a significant source of contamination (Arthur et al., 2014; Visvalingam et al., 2016; Yang et al., 2017a; Yang et al., 2015b). Some *E. coli* can survive the routine daily cleaning and sanitization of equipment (Yang et al., 2017b), which could be a result of poor sanitation between shifts or biofilm formation (Yang et al., 2018). Thus, the difference in heat resistance in the two trim *E. coli* populations could be related to the cleaning regime used at different plants. Whether this is indeed the case warrants further study. No difference between the two carcass populations or the two trim populations in their response to alkaline or between the four populations to acid treatment was observed. These findings suggest that the three commonly used decontamination interventions are unlikely to select for an *E. coli* population with increased frequency of resistance or cause cross-protection/sensitization of *E. coli*. However, since the bacteria in this study were not subjected to experiments in which they were sequential exposed to treatments, this study cannot reveal whether there is a protective effect due to adaptation following prior stress

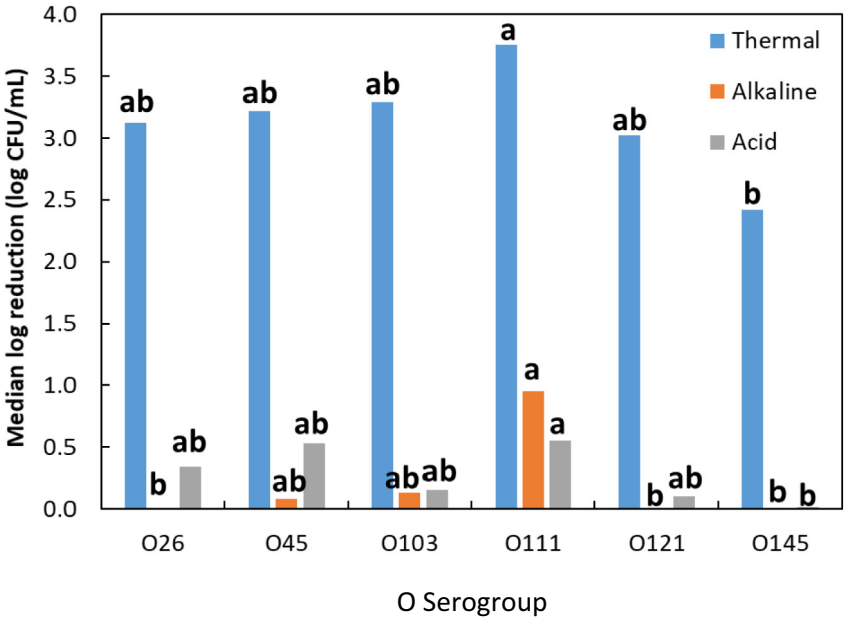

Fig. 4. Median reductions of the Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121 and O145 after thermal (60 °C for 2 min), alkaline (pH 11 for 2 h), and acid (pH 2.9 for 1 h) treatments. Letters denote comparisons between different serogroups within a treatment. Reductions that do not share a common letter are significantly different ($p < 0.05$).

exposure as described in previous studies (Garren et al., 1998; Samelis et al., 2005).

Within the populations of STEC strains isolated from humans, cattle or beef, individual strains varied significantly in their response to heat, acid or thermal treatment. The response of STEC to heat and acid was not isolation source related. The finding that there is no significant difference between the response of STEC O157 and STEC non-O157 to heat or lactic acid treatment is in agreement with the results in previous studies conducted on inoculated beef (Kalchayanand et al., 2015; Kalchayanand et al., 2012). Strain variability of STEC to stresses and source/serogroup-independent response to heat and acid treatment has been reported in studies where response to heat treatment, among other treatments, was investigated (Lee et al., 2012; Liu et al., 2015). Unlike their response to acid and heat treatment, STEC strains from cattle had a higher median log reduction after alkaline treatment than those from human, 0.6 vs 0.1 log CFU/mL, which could account for the difference in response observed between STEC O157 and STEC non-O157 to alkaline treatment, as the former group consists mostly of strains isolated from cattle. For *Salmonella*, no correlation between heat, acid, or alkaline resistance and isolation origin or serotype was evident. The variability of *Salmonella* heat resistance (2.1–5.0 log CFU/mL) was lower than acid (0.5–5.0 log CFU/mL) or alkaline (0.0–5.0 log CFU/mL) resistance. Low variability of *Salmonella* heat resistance compared to acid resistance, and compared to other organisms has been reported (den Besten et al., 2018; Lianou and Koutsoumanis, 2013). Information on alkaline response of STEC or *Salmonella* in the literature is meager. We are aware of only one relevant study, Álvarez-Ordóñez et al. (2013) in which the response of 12 *E. coli* strains including 10 STEC to alkaline treatment at 11.0 was evaluated. $D_{pH11.0}$ values varied from 49 to 139 min. The beef and human STEC strains assessed in this study appear to be more resistant to alkaline treatment than those reported by Álvarez-Ordóñez et al. (2013) with no significant reduction in cell counts observed for most strains after incubation at the same pH level for 2 h. However, care should be taken in making such comparisons as differences in observed sensitivity to stresses may reflect the choice of strains used in the study, particularly when relatively small numbers are used. The results will also be dependent upon the experimental system used, for example, in the experiments of Alvarez-Ordóñez et al. the cells were harvested in stationary phase and suspended in phosphate buffered saline, thus differing from this study in both growth phase and the availability of substrates for active protective mechanisms and the presence of complex organic molecules.

Compared to STEC and *Salmonella*, generic *E. coli* had a lower median log reduction after heat treatment. The difference in mean log reduction of generic *E. coli* and STEC was 0.4 log unit. Differences up to 0.3 log unit in mean reductions of total *E. coli* and *E. coli* carrying stx_1, stx_2 or *eae* genes in cattle hide enrichment cultures, as determined by real-time PCR, have been reported (Yang et al., 2014). Higher heat sensitivity of *Salmonella* was noted when five *Salmonella* strains were compared against nine strains of *E. coli* in phosphate buffered saline at 60 °C (Cabrera-Diaz et al., 2009). The findings of this study on relative heat resistance of generic *E. coli*, STEC and *Salmonella* are in line with published accounts and suggest total *E. coli* counts can be indicative of the potential for STEC and *Salmonella* survival of heat treatment.

Enteric bacteria are in general acid tolerant as they can encounter low gastric pH between 1.5 and 3.0, and short-chain fatty acids in the small intestine (Kanjee and Houry, 2013). Even so, different members of enteric bacteria may have different mechanisms for acid resistance and consequently vary in acid resistance. *Shigella flexneri*, and *E. coli*, but not *Salmonella* Typhimurium, were reported to survive exposure to pH 2.5, acidified with HCl (Gorden and Small, 1993; Lin et al., 1995), though the survival of *E. coli* varied between strains. All three species share general acid tolerance systems, but some strains of *E. coli* possess several acid resistance systems that are not found in *Salmonella* (Lin et al., 1995). Cabrera-Diaz et al. (2009) tested lactic acid resistance of nine *E. coli* and five *Salmonella* strains and found the *Salmonella* strains were, in general, less acid resistant than the *E. coli* strains. The outer membrane of *Salmonella* is more prone to lactic acid permeabilization than that of *E. coli* (Alakomi et al., 2000). It is then not surprising that *Salmonella* strains were reduced by > 3 log units more than *E. coli* when acidified with lactic acid to a pH of 2.9, in this study. Higher acid resistance was more frequent among the STEC than the generic *E. coli* strains in this study. A study of Grauke et al. (2003) found a different level and pattern of acid resistance for generic *E. coli* and *E. coli* O157 in cattle fed with different diets. In a previous study, we tested the response to lactic acid at pH 3.6 of different *E. coli* populations in cattle hide enrichment cultures (Wang et al., 2014). The finding of that study was that STEC harboring stx_2 or *eae* genes were more likely to be acid resistant than generic *E. coli* or STEC carrying stx_1. This difference between generic *E. coli* and STEC in acid resistance could be a result of the difference in the structure and the extent of expression of the lipopolysaccharide including O-polysaccaride on the cells surfaces (Barua et al., 2002; Martinić et al., 2011). Whether STEC, particularly STEC O157, is more acid resistant than *E. coli* in general has long been a

matter of debate in the literature. This inconsistency is due, in part, to the fact that many studies infer population behavior based on data from a small number of strains. Nevertheless, the findings of this study suggest that caution should be exercised when using generic *E. coli* counts as an indicator for assessing the survival potential of STEC following organic acid decontaminating treatments at abattoirs. The relative response to alkaline treatment of generic *E. coli*, STEC and *Salmonella* was similar to that of acid treatment.

The populations of generic *E. coli*, STEC and *Salmonella* differed in the correlation of their response to different stresses, with a moderate correlation between acid and alkaline response of *Salmonella*, no correlation between any pairs of the three treatments for generic *E. coli*, and moderate correlations between all three pairs of treatments for STEC noted. Information on correlation between different stress resistance for either *Salmonella* or *E. coli* is scarce. No correlation between acid resistance and heat resistance was found in a study in which 60 *Salmonella* strains were assessed for their heat and acid resistance (Lianou and Koutsoumanis, 2013). Álvarez-Ordóñez et al. (2013) reported a significant positive correlation between STEC resistance to heat and to alkaline stress, but no correlation of the resistance to the other stresses used in this study of ten STEC and two non-pathogenic *E. coli* strains. The different correlation patterns observed in the generic *E. coli*, *Salmonella* and STEC populations might indicate differences in their resistance mechanisms.

The findings of this study show that the three commonly used decontamination interventions would be unlikely to cause selection for increased frequency of resistance in *E. coli*. The response of *Salmonella* to the three treatments is not dependent upon the isolation source, while STEC resistance to alkaline treatment differed from this pattern. Total *E. coli* counts can be indicative of the survival potential of *Salmonella* in response to heat, acid and alkaline treatment. As for STEC, total *E. coli* counts can be a reliable indicator of the survival potential following heat treatment, but caution should be exercised when using total *E. coli* counts as an indicator for assessing the efficacy of organic acid- or alkaline-based interventions as it may lead to an underestimation of the survival potential of STEC. However, these conclusions are based upon comparison of the median reduction observed across a population of strains, and are relevant to the use of total *E. coli* counts as hygiene indicator. The results of this study are not contradictory to the established use of well characterized strains of generic *E. coli* as surrogates for STEC, including STEC O157, in-plant validation of interventions to reduce pathogen contamination (Keeling et al., 2009; Niebuhr et al., 2008).

This study also found variability of several orders of magnitude between strains of the three populations (generic *E. coli*, STEC and *Salmonella*) when subjected to acid, alkaline or thermal treatment. In designing experiments to assess the effect of decontamination treatments on artificially inoculated bacteria, care should be taken to ensure that the strains selected, whether pathogens or surrogates, do not represent the least resistant proportion of the population, as this could overestimate treatment lethality.

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