



Research Paper

Differences in *Salmonella* Serovars Response to Lactic Acid and Peracetic Acid Treatment Applied to Pork

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ABSTRACT

Pathogen control in the meat industry relies on the effectiveness of postharvest interventions in reducing microbial populations. This study investigated differences in the survival of *Salmonella* serovars when exposed to organic acids used as antimicrobials on raw pork meat. Seven serovars were included in this study (*S. Newport*, *S. Kentucky*, *S. Typhimurium*, *S. Dublin*, *S. Heidelberg*, *S. Infantis*, and *S. Enteritidis*). Multistrain serovar cocktails were prepared and tested against lactic acid (LA) and peracetic acid PAA at two concentrations, LA 2 and 4% and PAA 200 and 400 ppm. Pork samples were assigned to each serovar, inoculated with 6.0 Log CFU/cm^2 *Salmonella* (one serovar at a time), and treated with the corresponding antimicrobials. A two-way analysis of variance was conducted to examine the effects of serovar and antimicrobial concentrations on *Salmonella* survival. A significant main effect of serovar was identified, indicating that *Salmonella* concentration and reduction rate were significantly affected by serovar. Similarly, a significant main effect of antimicrobials was observed, suggesting that the treatment types impacted *Salmonella* concentration and reduction rate. However, the interaction effect between serovar and antimicrobial was not significant. Posthoc comparisons indicate that PAA 400 ppm is more effective at reducing *Salmonella* concentrations and that *S. Dublin* may be more susceptible than *S. Newport* to antimicrobial sprays. Additionally, under PAA exposure, only *S. Dublin*, *S. Kentucky*, and *S. Heidelberg* showed statistically significant differences ($P < 0.05$) compared with the control, indicating that these three serovars are more susceptible to PAA treatments than the rest. The behavior of different *Salmonella* serovars under stress conditions can give us an insight into how these pathogens survive processing.

The Centers for Disease Control and Prevention (CDC) has estimated that *Salmonella* causes 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year (CDC, 2024a), being the leading cause of foodborne diseases and outbreaks in the country (Popa & Popa, 2021). While *Salmonella* outbreaks are often linked to meat and poultry, the pathogen can contaminate a wide variety of food and easily survive and adapt to harsh environments (Dubois-Brissonnet, 2012). Government, research institutions, and industry are continuously engaged in researching and implementing strategies to mitigate the growth and spread of *Salmonella* during meat and poultry production (FSIS, 2022). These measures, known as postharvest interventions, are applied to animal carcasses after slaughter and meat products during processing (Sohaib et al., 2016). Nonetheless, the most effective strategy for postharvest interventions is implementing a multihurdle approach, as no method alone can eradicate pathogens from food products entirely (Mogren et al., 2018). A common application consists of organic acids sprayed on carcasses

postvisceration (Nkosi et al., 2021), broadly known to reduce bacterial concentrations without negatively impacting product quality (Abedi & Hashemi, 2020; Rossi et al., 2023). Examples of organic acids include lactic acid (LA) and peracetic acid (PAA). The Food and Drug Administration (FDA) has classified them as Generally Recognized as Safe (GRAS), indicating that they can be used freely in food production without posing any risk for human consumption (FDA, 2023).

The bacterial reduction effect of LA relies on its ability to permeate through the cell wall, release protons, and subsequently lower intracellular pH. LA is widely used in meat slaughtering facilities, and it is typically sprayed on food-animal carcasses postvisceration at concentrations of 2–4% (Silano et al., 2018). Similarly, PAA is another alternatively used to treat food-animal carcasses after slaughter. PAA has been approved by GRAS guidelines at concentrations of 0.005–2% (FSIS, 2023). Unlike LA, PAA acts as an oxidizing agent that targets the negatively charged wall of Gram-negative bacteria to scavenge electrons. Once inside the cell, it damages functional properties

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and cellular DNA (Aalto et al., 2024; Buschini, 2004). LA and PAA have been shown to reduce bacterial concentrations in various food matrixes and are regularly used in food production (Barcenilla et al., 2022; Bertram et al., 2019; Wang et al., 2020).

Despite the numerous mitigating strategies implemented by the industry, *Salmonella* outbreaks continue to occur. The latest Foodborne Diseases Active Surveillance Network report indicates an incidence of 16.3 per 100,000 in 2022, 1.88 times higher than in 2021 (CDC, 2024b). The increased incidence of *Salmonella* infections is thought to be attributed to serovar virulence, infectious dose, and serovar-host association (Cheng et al., 2019). Various studies have assessed protein expression and adaptation in acid-stressed *Salmonella* (Burin et al., 2014; Foster, 1991; Groisman et al., 2021). However, the limitations of some studies are related to the fact that their treatments are tested in suspension and fail to mimic food processing environments.

On the other hand, challenge studies that mimic food processing environments often evaluate an intervention by combining strains of several serovars in a bacterial cocktail (Calle et al., 2021). While that provides a representative effect of an antimicrobial treatment on various types of bacteria, identifying differences between *Salmonella* serovars' survival may explain potential challenges in *Salmonella* reduction. Serovar pathogenicity and infectious dose variations have been demonstrated at molecular and genetic levels (Cheng et al., 2019). This suggests that serovar-specific responses to antimicrobial treatments may also occur. Consequently, this study examines the response of seven *Salmonella* serovars —S. Newport, S. Kentucky, S. Typhimurium, S. Dublin, S. Heidelberg, S. Infantis, and S. Enteritidis—to LA and PAA, two commonly employed interventions in raw meat. The main goal is to assess *Salmonella*'s overall survival after antimicrobial treatments by testing if the target antimicrobial exhibits broad-spectrum or variable efficacy across the serovars.

Materials and methods

Strain selection. Seven *Salmonella* serovars were chosen for this study, consisting of Dublin, Enteritidis, Infantis, Kentucky, Typhimurium, Heidelberg, and Newport. A total of 20 strains were tested in this study, including two *S. Enteritidis* and three of all other serovars (Table 1).

Inoculum preparation. Seven *Salmonella* serovar solutions were prepared. Bacterial cultures were stored at -80°C with a cryoprotectant, and before each experiment, strains were recovered and freshly grown. A loopful of each frozen isolate was transferred to a test tube containing Tryptic Soy Broth (TSB) (BDTM, Franklin Lakes, NJ) and incubated at 37°C for 24 h. Following incubation, strains of the same serovar were combined by transferring equal aliquots of each to a prelabeled sterile tube and homogenizing. The original concentration of each serovar mix was tested by spread plating onto TSA (Remel, Lenexa, KS). Bacterial cocktails were freshly prepared for each biological replicate.

Antimicrobials preparation. Desired concentrations of LA (88% Lactic Acid, Lab Alley, Austin, Texas) and PAA (15% Peracetic Acid, Lab Alley, Austin, Texas) were prepared on the day of the experiment. The formula $C_1V_1 = C_2V_2$ was applied to prepare the mixtures and reach the desired concentrations. The required volume of the organic acids was then diluted in sterile water. Two concentrations of LA (2 and 4%) and PAA (200 and 400 ppm) were selected for this experiment based on the concentrations frequently used by the industry and allowed in meat processing (FSIS, 2021). The pH of the antimicrobial solutions was recorded after preparation using an Accumet® Basic AB15 pH meter (Fisher Scientific, Waltham, MA).

Antimicrobial spray treatment. Pork chop samples were purchased from a local grocery store and maintained at 4°C for no longer than one day prior to the experiment. Only lean meat was used.

Table 1
Salmonella strains and isolation source

Isolate ID*	Organism	Source of Isolate
R8-7251	<i>Salmonella enterica</i> ser. Dublin	dairy cattle feces
R9-3232	<i>Salmonella enterica</i> ser. Dublin	dairy cattle feces
FSIS-005	<i>Salmonella enterica</i> ser. Dublin	raw intact beef
DR-0862	<i>Salmonella enterica</i> ser. Enteritidis	poultry meat
DR-129C2	<i>Salmonella enterica</i> ser. Enteritidis	poultry feces
DR-0857	<i>Salmonella enterica</i> ser. Infantis	poultry meat
DR-0761	<i>Salmonella enterica</i> ser. Infantis	beef cattle feces
DR-0728B	<i>Salmonella enterica</i> ser. Infantis	swine feces
DR2-077C5	<i>Salmonella enterica</i> ser. Kentucky	poultry meat
ATCC 9263	<i>Salmonella enterica</i> ser. Kentucky	unknown
FSIS-003	<i>Salmonella enterica</i> ser. Kentucky	raw intact beef
DR2-165C5	<i>Salmonella enterica</i> ser. Typhimurium	poultry meat
ATCC 14028	<i>Salmonella enterica</i> ser. Typhimurium	chicken
FSIS-004	<i>Salmonella enterica</i> ser. Typhimurium	raw intact chicken
BAA-172 (8326)	<i>Salmonella enterica</i> ser. Heidelberg	unknown
R9-5495	<i>Salmonella enterica</i> ser. Heidelberg	poultry facility
S5 0448	<i>Salmonella enterica</i> ser. Heidelberg	human
ATCC 27869	<i>Salmonella enterica</i> ser. Newport	human
DD707	<i>Salmonella enterica</i> ser. Newport	unknown
ATCC 6962	<i>Salmonella enterica</i> ser. Newport	human

* Isolate ID refers to internal laboratory identification.

Samples were cut into 2×2 cm squares using a sterile scalpel and placed in an empty Petri dish. The Petri dishes containing samples were labeled accordingly to indicate the serovar and treatment (LA 2%, LA 4%, PAA 200 ppm, or PAA 400 ppm). Pork samples were surface inoculated with 20 μL of the bacterial cocktail to reach a target concentration of ca. 10^6 CFU/cm² and spread evenly using a sterile 1 μL disposable loop. A nonantimicrobial treated control was used for each serovar, which also served to estimate the initial concentration of the bacterial serovar cocktail attached to each sample. Following inoculation, the samples were placed under refrigeration (4°C) for 20 min. to allow bacterial attachment. After the attachment period, samples were treated immediately with their assigned antimicrobial treatment. Samples were sprayed at a 4 mL/cm² rate using a wide-mouth all-angle spray bottle. Spray bottles were calibrated before each experiment by spraying the antimicrobial concentration directly onto an empty beaker placed on a scale. Volumes were weighed, drawn up using a 1 mL pipette, and verified on a 10 mL graduated cylinder. Each sample was placed 15 cm from the edge of the biosafety cabinet, and sprayers were held at a 45° angle. All samples were treated with the antimicrobials for 30 min contact time before enumeration. Three biological repetitions (one technical replicate) were performed for each of the four treatments, which included two antimicrobials and two concentrations per each, along with a negative control (untreated sample). These experiments were carried out for each of the seven *Salmonella* serovars surveyed in this study.

Each treated pork sample was placed into a filtered Whirl-Pak bag with buffered peptone water (BPW) (Remel, Lenexa, KS) for a 1:10 dilution. Samples were homogenized at 230 rpm for 2 min using a Bag Mixer (Interscience Woburn, MA). Posthomogenization, serial ten-fold dilutions were prepared with BPW and thoroughly mixed. For the enumeration, samples were spread plated in duplicate onto Double Modified Lysine Iron Agar (DMLIA) (HIMEDIA®, Kennett Square, PA) agar and incubated at 37°C for 24 h; colonies were counted, the CFUs were estimated, and results were Log-transformed to report as Log CFU/cm². To measure the speed at which the microbial populations were inactivated, the reduction rate was calculated by taking the natural Log (ln) of the change in concentration after 30 min of exposure to each treatment type. The following formula was applied:

$$k = \frac{\ln\left(\frac{N_1}{N_2}\right)}{t_2 - t_1}$$

where k represents the rate constant, N^1 is the initial microbial concentration, N^2 is the final microbial concentration, and t_1 and t_2 are the corresponding time points (0 and 30 min).

Statistical analysis

All statistical analyses were conducted in RStudio (version 4.3.3). To explore how treatment type and serovar affect *Salmonella* concentration and serovar, a two-way analysis of variance (ANOVA) was performed. Similarly, a two-way ANOVA was also used to assess the effect of treatment type and serovar on the rate of reduction. Standard parametric assumptions were explored and met. In the model, antimicrobial (LA or PAA), antimicrobial concentration (2%; 4% or 200 ppm; 400 ppm), and serovar were considered possible variables influencing overall bacterial reduction. Additionally, a Tukey HSD posthoc was conducted for each statistically significant interaction observed to explore differences in each group. An alpha value of 0.05 was considered statistically significant. Three biological repetitions of each experiment were conducted.

Results

Statistical analysis from the two-way ANOVA revealed a significant relationship between *Salmonella* concentration and treatment type ($P < 0.05$) as well as *Salmonella* concentration and serovar ($P < 0.01$). However, no significant interaction effect of treatment type and serovar on *Salmonella* concentrations was observed ($P > 0.05$). Similarly, for the reduction rate, the two-way ANOVA results indicate a significant relationship between treatment type and rate ($P = 0.00000636$) and rate and serovar ($P < 0.05$). No significant interaction effects between treatment type and serovar were observed on the rate of reduction ($P > 0.05$).

Relationship between *Salmonella* survival and treatment type. The average *Salmonella* concentration by each treatment and results from the posthoc analysis are depicted in Figure 1. The concentration of *Salmonella* after LA 2% treatment was 6.01 Log CFU/cm², equivalent to a 0.24 Log CFU/cm² reduction. The concentration of *Salmonella* after LA 4% treatment was 5.86 Log CFU/cm², corresponding to a 0.39 Log CFU/cm² reduction. Compared to the nontreated controls, the overall reduction achieved by LA 4% was statistically significant

($P < 0.05$); however, no statistically significant ($P > 0.05$) differences were observed between LA 2% and LA 4%. On the other hand, PAA 200 ppm achieved an average reduction of 0.47 Log CFU/cm², while the overall reduction by PAA 400 ppm was 0.68 Log CFU/cm². Compared to the control, both concentrations of PAA were statistically significant ($P < 0.05$); however, no statistically significant ($P > 0.05$) differences were observed between PAA concentrations. Differences between antimicrobial treatments were observed, as PAA 400 ppm had a significantly greater ($P > 0.05$) bacterial reduction than both LA 2% and LA 4%.

The reduction rate by each treatment can be seen in Figure 2. The reduction rate constant k represents units of reciprocal time (per minute). Therefore, a higher k indicates a faster rate of reduction. Overall, PAA had a faster rate of reduction than LA, which can be confirmed with the upward trend in Figure 2. For LA, the rate of reduction did not statistically differ between the two concentrations tested ($P > 0.05$). On the other hand, for the PAA treatments, PAA 400 ppm had a rate that was statistically higher than PAA 200 ppm. Statistically significant differences ($P < 0.05$) were observed between both PAA concentrations and LA 2%; however, only PAA 400 ppm had a statistically higher rate than LA 4%. Overall, PAA 400 ppm achieved a reduction rate that was significantly faster than the other three treatments.

Relationship between *Salmonella* survival and serovar. The overall reduction of each serovar after all four treatments was assessed, as shown in Figure 3. Some serovar-dependent reduction ($P < 0.05$) was observed. Statistically significant differences between serovars were observed between *S. Dublin* and *S. Heidelberg* ($P < 0.05$) and *S. Dublin* and *S. Newport* ($P < 0.05$). Compared to the other serovars, the antimicrobial treatments achieved a concentration of *S. Dublin* of 5.66 Log CFU/cm², 0.607 Log CFU/cm² lower than the nontreated control. *S. Heidelberg* presented the second greatest concentration at 5.89 Log CFU/cm², 0.543 Log CFU/cm² lower than the untreated control. On the other hand, *S. Newport* had the lowest reduction after treatment. Counts were at 6.05 Log CFU/cm², with a 0.311 CFU/cm² reduction. To ensure that differences were not due to variations between the initial attachment of each serovar to the sample surfaces, a one-way ANOVA was conducted, which demonstrated no significant differences ($P > 0.05$) between controls. Therefore, the bacterial attachment level on the surface was not serovar-

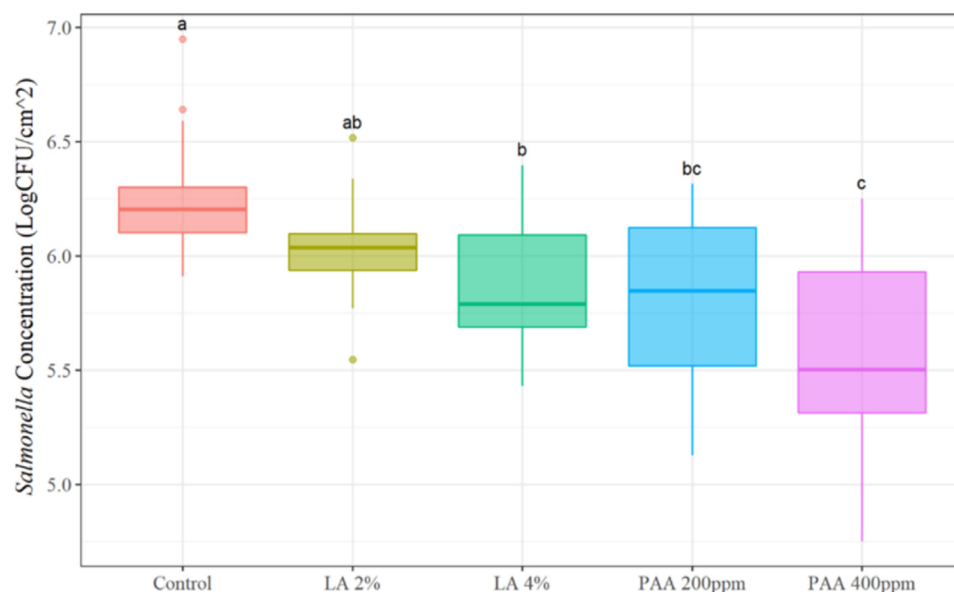


Figure 1. *Salmonella* concentrations after treatment exposure. The nontreated control can be seen to the far left, followed by the treatment types. Statistically significant differences are represented with different lowercase letters.

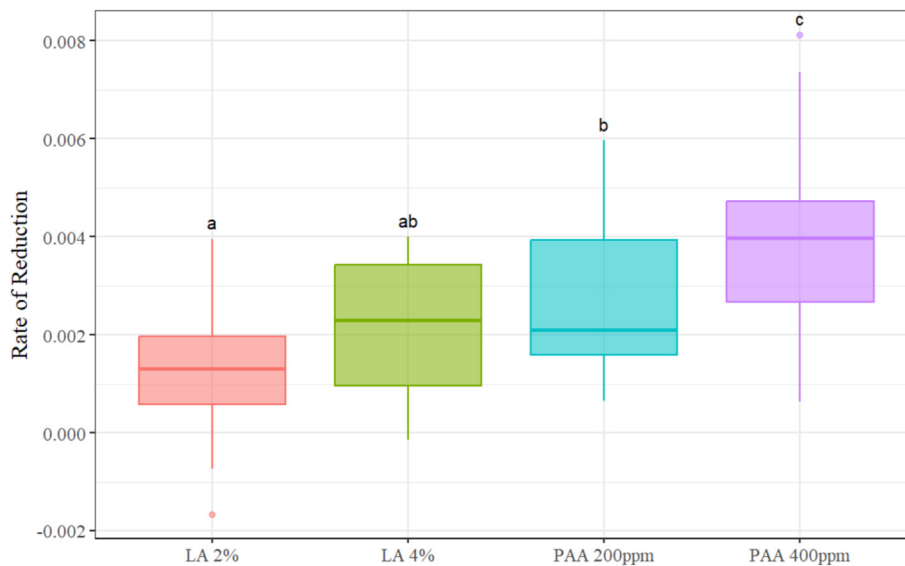


Figure 2. Reduction rate of *Salmonella* after treatment exposure. Statistically significant differences are represented with different lowercase letters.

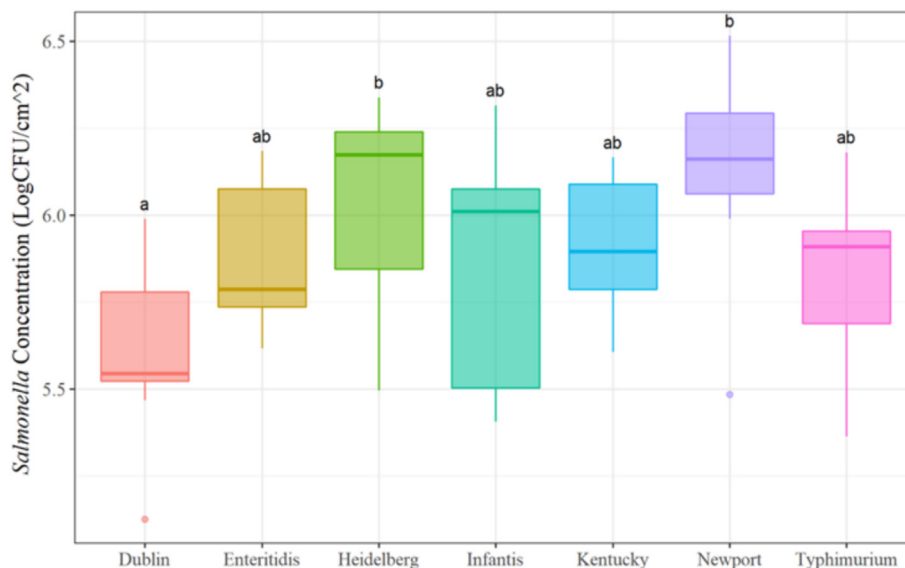


Figure 3. *Salmonella* concentrations posttreatment by serovar. The relationship of the cumulative *Salmonella* concentration of each serovar after treatments. Statistically significant differences are represented with different lowercase letters.

dependent, and similar attachment concentrations were achieved for all serovars in the treated samples, indicating that assumptions can be made about the overall *Salmonella* reduction. The overall combined reduction with the four treatments had a cumulative reduction between 0.311 and 0.607 Log CFU/cm², which achieved a concentration that significantly varied between serovars.

When the cumulative reduction rate after all treatments was analyzed, a significant relationship was found between serovar and reduction rate. Statistically significant differences were observed between *S. Dublin* and *S. Kentucky*. *S. Kentucky* had a significantly faster ($P < 0.05$) rate than *S. Dublin*. Moreover, there was a tendency for *S. Newport* and *S. Enteritidis* to have a faster rate than *S. Dublin*. The overall reduction rate of each serovar is depicted in Figure 4. *S. Dublin* and *S. Infantis* tended to have a faster rate of reduction, which could be responsible for the lower *Salmonella* concentrations, as seen in Figure 3.

Interaction effect of treatment type and serovar on *Salmonella* survival. No significant interaction effect of treatment type and serovar on *Salmonella* concentration or reduction rate was observed ($P > 0.05$). This indicates that survival of *Salmonella* serovars is not dependent on the level of antimicrobials at the concentration tested (treatment type). The results for *Salmonella* concentration can be observed in Figure 5, which depicts the interaction between antimicrobial type and *Salmonella* counts by serovar type with 95% CI. The continuous downward trend can still be observed where PAA 400 ppm achieved greater bacterial reductions; however, 95% CI overlapped for all serovar types, indicating no statistically significant relationship between serovar at each treatment type ($P > 0.05$). However, a trend was observed at PAA 200 ppm and PAA 400 ppm, where lower concentrations of *S. Dublin* were recovered after treatment compared to the other serovars treated. Although no significant differences were observed between serovars for the LA treatments, there was a higher

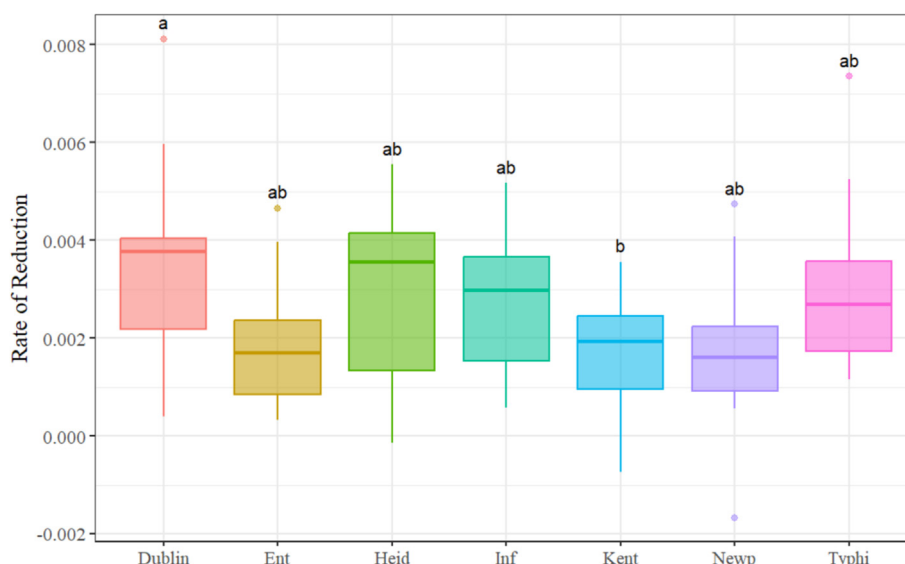


Figure 4. Cumulative reduction rate of *Salmonella* serovars after treatment exposure. Statistically significant differences are represented with different lowercase letters.

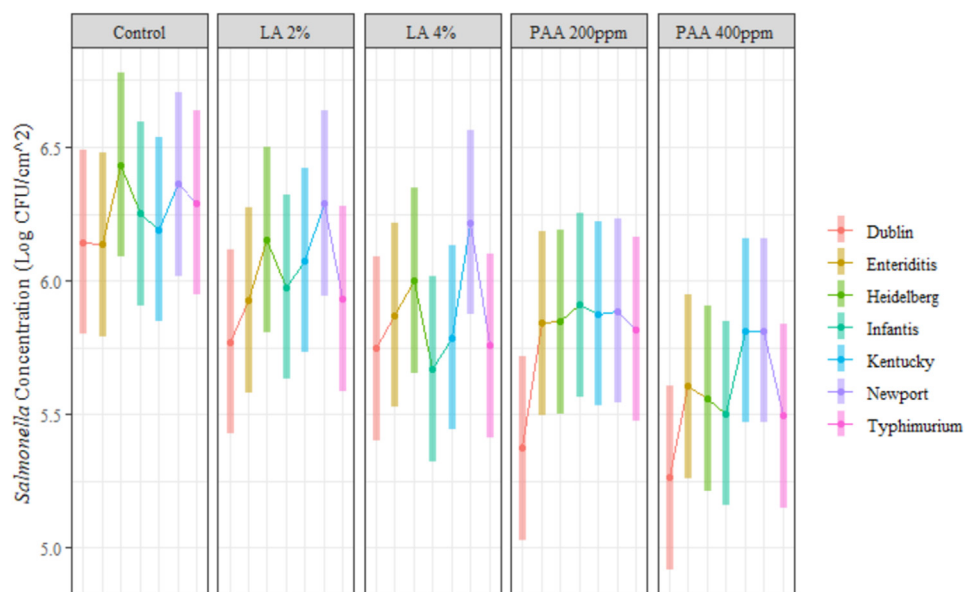


Figure 5. 95% CI contrasts between *Salmonella* serovars after each treatment. Overlapping 95% CI bars indicate no statistically significant difference between serovars.

concentration of *S. Newport* and a lower concentration of *S. Dublin* and *S. Typhimurium*, both LA 2% and LA 4%. However, there were no significant differences ($P > 0.05$) between the concentration of the samples treated with LA and the controls for all serovars. On the other hand, for the PAA treatments, significant differences ($P < 0.05$) were observed between the control and *S. Dublin* at both 200 ppm and 400 ppm. Moreover, significant differences ($P < 0.05$) between control and treatment at PAA 400 ppm were also found for *S. Heidelberg* and *S. Kentucky*, indicating that PAA may be more effective at reducing *S. Dublin*, *S. Heidelberg*, and *S. Kentucky*. *Salmonella* counts after each treatment can be seen in Table 2.

The results for *Salmonella* reduction rate are presented in Figure 6, which shows the interaction between antimicrobial type and the reduction rate by serovar type and 95% CIs. The continuous upward trend can still be observed where PAA 400 ppm achieved a faster

reduction rate, and LA 2% achieved a slower reduction rate. The 95% CI of the serovar reduction rate overlaps for all treatments, indicating no statistically significant interaction between serovar at each treatment type ($P > 0.05$). A statistically significant difference ($P < 0.05$) can be observed for *S. Heidelberg* between treatment LA 2% and PAA 400 ppm, where the PAA treatment generated a faster reduction rate (Table 3).

Discussion

The primary aim of this study was to investigate whether the destruction of *Salmonella* by LA and PAA is serovar-dependent. Specifically, we sought to identify differences in *Salmonella* reductions following treatment with two antimicrobials at varying concentrations while establishing if any relationships between *Salmonella* reduction

and treatment type are serovar dependent. Both LA and PAA are commonly used antimicrobial interventions for food product decontamination (Loretz et al., 2010; Zoellner et al., 2018). In the meat industry, LA is recognized as a safe, natural decontaminant as it is produced by lactic acid bacteria during fermentation (Snijders et al., 1985). PAA is also approved and well-accepted as a postharvest intervention in

Table 2
Concentrations of *Salmonella* serovars after each treatment

Serovar	Treatment	<i>Salmonella</i> Concentration (Log CFU/cm ²)	Standard Deviation
Dublin	LA 2%	5.771	0.223
Dublin	LA 4%	5.747	0.218
Dublin	PAA 200 ppm	5.373	0.215
Dublin	PAA 400 ppm	5.263	0.445
Enteritidis	LA 2%	5.926	0.153
Enteritidis	LA 4%	5.871	0.271
Enteritidis	PAA 200 ppm	5.842	0.257
Enteritidis	PAA 400 ppm	5.604	0.458
Heidelberg	LA 2%	6.153	0.195
Heidelberg	LA 4%	6.001	0.438
Heidelberg	PAA 200 ppm	5.847	0.329
Heidelberg	PAA 400 ppm	5.559	0.411
Infantis	LA 2%	5.976	0.16
Infantis	LA 4%	5.669	0.353
Infantis	PAA 200 ppm	5.911	0.462
Infantis	PAA 400 ppm	5.502	0.329
Kentucky	LA 2%	6.075	0.097
Kentucky	LA 4%	5.787	0.073
Kentucky	PAA 200 ppm	5.875	0.259
Kentucky	PAA 400 ppm	5.813	0.293
Newport	LA 2%	6.29	0.227
Newport	LA 4%	6.217	0.159
Newport	PAA 200 ppm	5.887	0.362
Newport	PAA 400 ppm	5.813	0.437
Typhimurium	LA 2%	5.933	0.145
Typhimurium	LA 4%	5.757	0.139
Typhimurium	PAA 200 ppm	5.818	0.416
Typhimurium	PAA 400 ppm	5.495	0.456

Salmonella concentration after each treatment expressed in Log CFU/cm² followed by the standard deviation. Treatments were LA = lactic acid and PAA = peracetic acid at two different concentrations.

produce, raw and cooked meat, and poultry. According to the USDA Food Safety Inspection Service (FSIS), it can be used in foods in concentrations of 0.005–2% (FSIS, 2023). In meat and poultry facilities, these treatments are used in carcasses, trim, and organs as an antimicrobial, and their application methods include a spray cabinet, dip tank, and hand spray pump (FDA, 2017). Due to the persistence of *Salmonella* in meat products, it is crucial to examine the effect of these interventions and to determine how individual serovars may adapt and survive such treatments (Ferrari et al. (2019)).

The overall reduction of *Salmonella* was low, with all the antimicrobial treatments achieving less of a 1 Log CFU/cm² reduction. Past studies have used antimicrobials such as LA to assess bacterial reduction, demonstrating reduction between 1 and 3 Log CFU/cm² (Beyaz & Tayar, 2010; Manzoor et al., 2020; Eastwood et al., 2021). Although those studies show a higher reduction rate, it is important to note that such reduction occurred when assessing total aerobic counts or generic *E. coli*. Furthermore, in instances where more significant reductions were achieved, the antimicrobial concentrations exceeded those currently permitted in food processing (Kumar et al., 2020). Conversely, studies employing acid sprays to treat meat and poultry surfaces against *Salmonella* have reported kill rates similar to the ones obtained in our study. Eastwood et al., 2021 assessed the reduction of *Salmonella* on skinless chilled pork and observed a reduction of LA at 2.5% and PAA at 400 ppm at room temperature and found an inactivation of 0.6 Logs and 0.5 Logs, respectively (Eastwood et al., 2021). Similarly, Bonilla et al., (2023) explored the use of an antimicrobial spray cabinet with PAA at 400 ppm and found reductions of *Salmonella* spp. on pork carcass cavities of 0.5–1.7 Logs. A higher reduction was achieved on pork carcass skin of 2.0–3.0 Logs (Bonilla et al., 2023). In contrast, Gonzalez et al., (2023) examined the effect of various antimicrobial treatments on pork skin samples with low (3–4 Log cm²) and high (6–7 Logs cm²) inoculation levels. Their results indicated that treatment with PAA at 400 ppm led to a reduction of 0.2 Log CFU/cm² in both high and low inoculation samples 24 h posttreatment. The study found that the reduction after 24 h of treatment and the reduction immediately after treatment were not significantly different (Gonzalez et al., 2023). Additionally, Kalchayanand et al., (2024) assessed spray treatments ranging from 130 to 400 ppm of PAA and observed a reduction of 0.2–0.3 Log of *Salmonella* on beef trim surfaces (Kalchayanand et al., 2024).

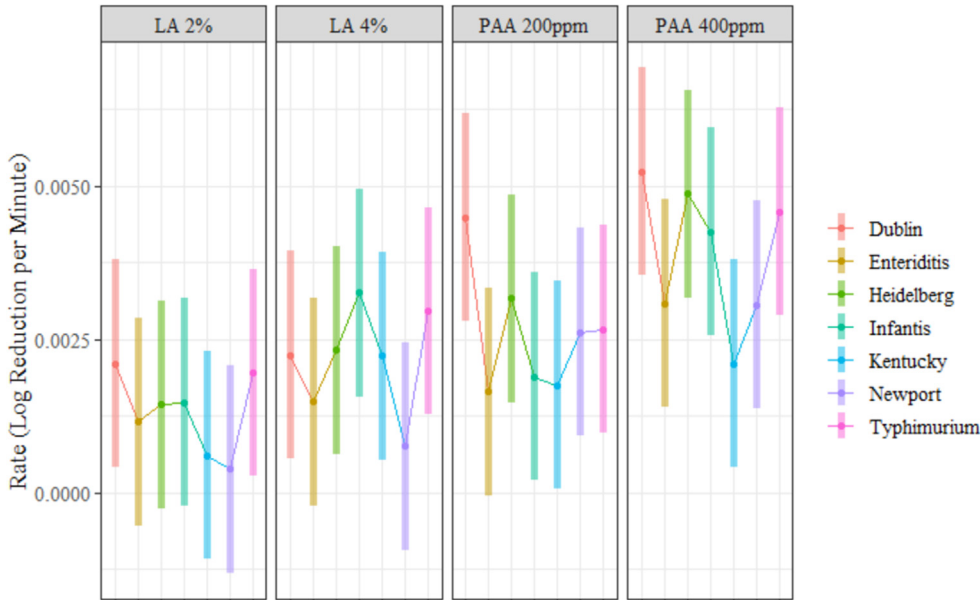


Figure 6. 95% CI contrasts between *Salmonella* serovars reduction rate after each treatment. Overlapping 95% CI bars indicate no statistically significant difference between serovars.

Table 3
Ph of antimicrobial solutions

Antimicrobial	Concentration	pH
Lactic Acid	2%	3.3 ± 0.20
Lactic Acid	4%	1.8 ± 0.26
Peracetic Acid	200 ppm	3.7 ± 0.44
Peracetic Acid	400 ppm	3.2 ± 0.41

Average of measurements per the solution before application to the samples.

Combining various methods has also been studied, as adding stressors simultaneously may increase the effectiveness of reducing *Salmonella*. Milillo et al., (2011) found that combining heat (50–60 °C) and organic acids can effectively reduce populations (Milillo et al., 2011; Eastwood et al., 2021). However, in their case, the acid treatments were tested in suspension, which can have a different effect than a spray application. Although the reduction rates of *Salmonella* as treated with LA and PAA are low, for most studies, the treatments showed statistically significant differences from the nontreated control. Furthermore, a limitation of *in-vitro* studies is the high concentration of the inoculum used for attachment to food surfaces before treatment. Most studies target ca. 6–8 Logs on surfaces before treatment, higher than the 1–3 Logs commonly observed upon product arrival into a facility (Bueno López et al., 2022; De Villena et al., 2022). In other words, higher concentrations of the bacterial population could make it harder for cell destruction. Moreover, statistically significant differences observed in *in-vitro* studies are challenging to translate into antimicrobial efficacy in food processing.

To our knowledge, this is the first published study investigating whether the reduction of *Salmonella* through chemical treatment is serovar-dependent under simulated industry conditions. A previous study established that not all *Salmonella* serovars produce an equal response to PAA treatments. Šovljanski et al. studied the effect of peracetic acid in suspension, as opposed to spray application, in *S. Enteritidis*, *S. Typhimurium*, *S. Derby*, and *S. Agona*. They found an increased sensitivity for *S. Enteritidis* and *S. Agona* compared to the other two serovars (Šovljanski et al., 2023). Their findings suggest that PAA can be selectively effective against some serovars, highlighting a variable sensitivity among different *Salmonella* serovars.

Our study did not find a significant interaction between serovar and the four treatments; however, there were some significant differences between control and PAA for *S. Dublin*, *S. Heidelberg*, and *S. Kentucky*, which demonstrate that PAA can be more effective at reducing concentrations of targeted serovars. Overall, PAA achieved a greater reduction of *Salmonella* concentrations, as can be seen in the downward trend in Figures 1 and 3. Specifically, PAA 400 ppm was more effective at reducing *Salmonella* than the other treatments; however, the reduction rate was <1 Log CFU/cm², indicating more effective interventions are needed to further reduce *Salmonella* concentrations.

One explanation is the adaptation to acid stress. Reports have primarily focused on *S. Typhimurium* and its acid tolerance response (ATR) (Pradhan & Devi Negi, 2019). The mechanism triggering the adaptation involves *S. Typhimurium* exposure to moderate acid stress, causing the synthesis of proteins that can sense and respond to acidification (Foster, 1991; Groisman et al., 2021; Wilmes-Riesenberg et al., 1996). In industry settings, the *Salmonella* ATR response can be induced when the pH of antimicrobial sprays or immersion tanks falls below sublethal levels (Lang et al., 2021). This can allow the pathogen to survive postharvest interventions. Moreover, an acid-adapted *Salmonella* can have a protected physiological state, rendering additional interventions in a multihurdle approach less efficient (Foley et al., 2013). Some serovar differences have been explored. For instance, Joerger et al. in 2012 found that *S. Kentucky* tends to be more acid-sensitive in the presence of acetic acid (Joerger et al.,

2012). Moreover, Etter et al. in 2019 performed RNA sequencing on *S. Heidelberg* isolates that were related to an outbreak and found increased stress tolerance and biofilm formation abilities (Etter et al., 2019). Although the current study found no serovar-dependent differences in reducing *Salmonella* populations on pork as individual treatment types, there were significant differences between some serovars and the controls at PAA concentrations. Therefore, continuing to study serovar differences can help identify unique genotypic and phenotypic traits that distinguish *Salmonella* serovars from each other. This can perhaps lead to policy changes and postharvest interventions focusing on specific serovars that have been highly associated with human illnesses. As *Salmonella* incidence remains high, an assessment of the current postharvest interventions needs to be carried out. Understanding serovar differences can lead to better decision-making, improved food safety, and increased public health.

CRedit authorship contribution statement

Mariana Fernandez: Writing – original draft, Methodology, Investigation, Formal analysis. **Alexandra Calle:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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