**Does Antibiotic Resistance Influence Shiga-Toxigenic *Escherichia coli* O26 and O103
Survival to Stress Environments?**

Running Title: STEC antibiotic-resistant against environmental stress

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ABSTRACT

The presence of antibiotic-resistant bacteria in the food supply chain is a major concern. There is a great need to revalidate the effectiveness of the intervention strategies commonly used in food processing environments for multidrug-resistant strains. The purpose of this study was to determine the survival of antibiotic-resistant O26 and O103 Shiga-toxin producing *Escherichia coli* (STEC) strains to lactic acid (2.5, 3.5, 5%), sodium hypochlorite (0.2, 0.5, 1 ppm free chlorine), and heat (60, 61, 62.5°C) treatments. Six strains from STEC serotypes O26 and O103 with three distinctive antibiotic-resistant profiles (susceptible, low and high resistance) were selected for the study. As expected, the rates of inactivation were faster at increasing biocide concentrations or temperature level. The Weibull model provided a better fit than the traditional log-linear model due to the appearance of nonlinear patterns. A 5% lactic acid treatment for 10 min reduced the population of all STEC strains by 5 log CFU/ml. Sodium hypochlorite treatment (1 ppm for 5 min) achieved a similar reduction. Thermal D values for O26 and O103 serotypes ranged from 0.37-2.09 and 0.37-1.71 min, respectively. No statistical differences ($P>0.05$) in tolerance were found between the two STEC serotypes to the different treatments. The antibiotic profile was not related to the ability to tolerate any of the treatments. Only the susceptible strain showed a relative higher resistance to the lactic acid and lower resistance to sodium hypochlorite treatment. These results would contribute to assess the risk for the presence of STEC strains in food processing environments.

Keywords: non-O157:H7 *E. coli* STEC, antibiotic resistance, predictive microbiology, sodium hypochlorite, heat, lactic acid.

1. INTRODUCTION

Non-O157:H7 Shiga toxigenic *Escherichia coli* (STEC) strains belong to a group of over 400 STEC serotypes with only a subset capable of producing illness in humans such as diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Croxen et al., 2013). Six non-O157 serogroups more commonly associated with outbreaks and severe illnesses (O103, O111, O121, O145, O26, and O45) are responsible of 270 hospitalizations per year in United States (Scallan et al., 2011). These serogroups were declared adulterants by the United States Department of Agriculture, Food Safety and Inspection Service (FSIS) in raw beef trimmings in 2011 (FSIS, 2011). According to the last foodborne disease outbreak annual report from the Centers for Disease Control (CDC), in 2014 the number and incidence of infections per 100,000 population of *E. coli* O157:H7 declined compared with 2006-2008 period (445 illnesses [0.92 incidence rate]) whereas it was significantly higher for STEC non-O157 (690 illnesses [1.43 incidence rate]) possibly due to an increase in the number of laboratories testing for Shiga toxin producing serotypes (CDC, 2015). Among these, O26 and O103 are the two most common serogroups causing almost 40% of all non-O157 infections reported (CDC, 2009; Brooks et al., 2005; Bosilevac and Koohmaraie, 2011).

There is a growing global concern on the presence of multidrug resistant bacteria in the food chain due to the use of antibiotics for therapeutics and growth promoters in feed for food animals. According to CDC estimation, each year approximately 2 million people develop serious illness and 23,000 people die due to infections with antibiotic resistant bacteria in US (CDC, 2013). Ultimately, the U.S. Food and Drug Administration (FDA) have released several rules to call on animal drug sponsors to restrict the use of antimicrobials related to growth promotion and bring more veterinarian oversight (FDA, 2013 and 2015).

Different interventions strategies (carcass sanitization, physical or chemical decontamination treatments for fresh meat) are currently adopted by the beef industry to control STEC. Different antimicrobials such as lactic acid, peroxyacetic acid, sodium chlorite, acidified sodium hypochlorite, potassium lactate are most widely used for a conventional control program to decontaminate meat (Geornaras et al., 2012a and b; Pittman et al., 2012). Among physical interventions, heat and high pressure treatments are the most common methods for pathogen inactivation (O'Bryan et al., 2006, Bajovic et al., 2012).

It is evident that wide and inappropriate use of antimicrobials to control pathogenic bacteria may pose a risk for the selection and dissemination of resistant flora. This process is known as 'cross-protection' when adaptation to one stress can confer increased resistance to other unrelated stresses (IFT, 2006). Scientists have studied the correlation between the use of sub-lethal concentrations of antimicrobials with the contribution on the expression and spread of antibiotic resistance mechanisms (Walsh et al., 2003; Potenski et al., 2003; McMahon et al., 2007; Capita et al., 2014) and heat tolerance (Flacaut et al., 1997). The reverse phenomenon to identify if antibiotic resistance provides any selective advantage to microorganisms for better survival and growth in an antimicrobial-challenged or other control environment has also been studied in a number of bacteria namely *Staphylococcus aureus*, *Salmonella* Newport, *Salmonella* Typhimurium and *E. coli* O157:H7 (Nostro et al., 2004; Fouladkhah et al., 2012, Geornaras et al., 2012b; Oscar et al., 2013). So far, to our knowledge, no research data are currently available on the association of multidrug resistance in non-O157 STEC and their ability to survive chemical and physical interventions.

The objective of the present study was to characterize the inactivation kinetics of STEC-O26 and STEC-O103 isolates with different antibiotic resistance profiles to different interventions used by the beef industry (antimicrobial wash and thermal processing).

2. MATERIALS AND METHODS

2.1. Selection of bacterial strains

A total of six non-O157 Shiga toxigenic *Escherichia coli* (STEC) strains belonging to serogroups O103 and O26 were used in this study. All strains of serogroup O26 belonged to serotype O26:H11 while two strains of the serogroup O103 belonged to the O103:H2 serotype and the other to serotype O103:H18 serotype (Table 1). *E. coli* O26 (30-2A-Ca and E25) and O103 serogroup strains (10-4aA and E68) were obtained from a dairy cattle farm surveillance study conducted earlier (Cho et al. 2006) Additional strains of O26 (O26-2) and O103 (O103-2) serogroups were obtained from USMARC (U.S. Meat Animal Research Center, USDA-ARS, Nebraska). All strains were selected based on previously identified antibiotic resistance profiles: high [resistant to eight antibiotics, High-O26 (30-2A-ca) and High-O103 (10-4aA)], low [resistant to two to three antibiotics, Low-O26 (E25) Low-O103 (E68)] and susceptible [no resistance observed, Sus-O26 (O26-2) and Sus-O103 (O103-2)]. The panel of antibiotics used for screening is shown in Table 1.

All bacterial strains from both serogroups were maintained as frozen (-80°C) culture in glycerol stock into tryptic soy broth (TSA) (Neogen Corp., Lansing, MI). All working cultures were made by streaking the frozen stocks onto TSA (Neogen Corp.) and stored at 4°C for two weeks. Prior to experiments, each strain was grown by selecting an isolated colony from TSA plates and inoculating into tryptic soy broth (TSB) (Neogen Corp.) and incubated at 37°C to obtain fresh overnight cultures.

2.2. Selection of control methods

The inactivation of antibiotic resistant versus susceptible STEC O26 and O103 strains were compared and characterized with three different control methods used extensively in the beef industry: lactic acid (certified ACS 85% w/w, Fisher Scientific, Fair Lawn, NJ), sodium hypochlorite (reagent grade, Sigma Aldrich Co., MO) and thermal treatments.

2.3. Effects of lactic acid (LA) treatment on STEC inactivation kinetics

The effects of LA treatment on the survival of non-O157:H7 STEC strains were studied in buffered peptone water (BPW, pH 6.5) at room temperature. STEC strains were freshly grown in TSB at 37°C for 18 h from working cultures to obtain overnight cultures. The cells were harvested by centrifugation at $10000 \times g$ for 10 min and washed twice with BPW (pH 7.2). The pellets were resuspended in BPW and diluted to achieve an initial concentration of $\sim 10^7$ CFU/mL. The actual concentrations of the STEC inocula were determined by plate counting method using TSA at 37°C for 18 h.

Three different LA solutions (2.5, 3.5 and 5% w/v) were prepared with sterile distilled water. Bacterial strains were incubated for 1 h at room temperature (25°C) for temperature equilibration. Three LA treatments (2.5%, 3.5% and 5%) were added to each STEC strain suspension (BPW, pH 6.5) to challenge the bacterial cells for up to 10 min at room temperature. In control groups, the volumes of LA were compensated by adding distilled water (the diluent of LA) in bacterial suspensions of BPW. Samples were collected at 0, 1, 2.5, 5, 7, and 10 min and immediately diluted in 10-fold serial dilutions into BPW (pH 7.8) to neutralize the pH (7.2 ± 0.2) prior to plating. Treated samples were plated (0.1 mL) on TSA plates and incubated at 37°C for 24-48 h. Viable cell counts were determined for treatments and control.

2.4. Effects of sodium hypochlorite (SH) treatment on STEC inactivation kinetics

The effects of SH treatment on the survival of non-O157:H7 STEC strains were studied in 0.9% NaCl suspension and treated with three concentrations (0.2, 0.5 and 1 ppm free chlorine) of sodium hypochlorite or control group for 0, 1, 2.5, 5, 7 and 10 min at room temperature. After treatment, cells were diluted into neutralizing buffer to stop the chlorine reaction prior to plating. Samples were taken, plated and counted similarly as described in previous section. Different Stock solutions were made by diluting sodium hypochlorite in sterile distilled deionized water. Free chlorine in stock solutions was measured by LaMotte chlorine test kit (LaMotte Company, MA).

2.5. Effects of heat treatment (HT) on STEC inactivation kinetics

The effects of the heat treatment on the STEC strains was studied by transferring 0.05 mL of the initial bacterial suspensions to 0.2 mL sterile flat-top cap tubes (Thermo Fisher Scientific, Inc., MA). The tubes containing the liquid of bacterial suspensions were warmed-up in a thermal cycler (T100 TM thermal Cycler, Bio-Rad Laboratories, Inc. CA), through direct contact with a hot metal plate, in a conductive heat setting. The tubes containing the samples were kept at room temperature before the treatment. One sample was taken before any treatment, as control (time 0). The tubes were placed in the micro-wells of the thermal cycler and sampling was obtained after 1, 2, 2.5, and 3 min for samples treated at 60°C, after 1, 2, 2.5, 2.75, and 3 min for samples treated at 61°C, and after 1, 1.5, 1.75, and 2 min for samples treated at 62.5°C. These time intervals were chosen to allow a viable bacterial count after each thermal treatment. The temperature of the suspension was measured with a Type-K beaded thermocouple (Traceable Expanded-Range Thermometer with Type-K beaded probe, Thermo Fisher Scientific, Inc., MA) and the exposure time was calculated when the sample reached the selected temperature (20 s at

60°C, 22 s at 61°C and 25 s at 62.5°C). After each treatment, the tubes were immediately placed in an ice-water bath for 1 min, and then moved back to room temperature. Samples were serially diluted in sterile saline (0.9% NaCl), plated on TSA and incubated at 37°C for 24-48 h.

2.6. Data analysis

All reported results were calculated by taking the average of two independent experimental replicates in which duplicate samples with duplicate counts were obtained at each time interval. The detection limit for bacterial counts was 1 log CFU/mL. Mean and standard deviations were determined using Microsoft Excel (Version 14.0; Microsoft, Corp; WA).

GInaFiT a freeware Add-in for Microsoft Excel was used to obtain the kinetic parameters to describe the effects of the different treatments (Geeraerd et al., 2005).

The traditional log-linear model developed by Bigelow and Esty (1920) was used to characterize the survival curves by using the following equation:

$$\text{Log}(N) = \text{Log}(N_0) - kt \quad \text{Eq. 1}$$

where N is the surviving cells after the treatment expressed as CFU/mL, N_0 the initial microbial population (CFU/mL), t is the holding time (min), and k is the kinetic constant (min^{-1}).

The kinetic parameter D (min) known as the decimal reduction time (time at certain temperature to reduce 90% or 1 log the initial microbial population) can be calculated as follows:

$$D = 2.3/k \quad \text{Eq. 2}$$

Models based on the Weibull distribution function have been used to describe the nonlinear inactivation patterns of different pathogens after processing interventions. Assuming that the resistance of the individual cells of a microbial population to a particular stress is governed by a Weibull distribution, Mafart et al., (2002) developed a Weibullian equation by using the following:

$$\text{Log}(N) = \log(N_0) - \left(\frac{t}{\alpha}\right)^\beta \quad \text{Eq. 3}$$

where N is the surviving cells after the treatment expressed as CFU/mL, N_0 the initial microbial population (CFU/mL), α is the time of the first logarithm decline for the microbial population (min), and β is the shape parameter. The β value gives an idea of the form of the curve; if $\beta > 1$, the curve is convex (it forms shoulders), if $\beta < 1$, the curve is concave (it forms tails), and if $\beta = 1$, the curve is a straight line and can be described by a linear model.

Statistical analyses were performed by analysis of variance (ANOVA) and Duncan's multiple range test for differences ($P < 0.05$) between control and treatment groups using SAS software (9.2; Statistical Analysis Systems, Cary, N.C., USA).

3. RESULTS AND DISCUSSION

The hypothesis evaluated in this study was to test if the extent of antibiotic-resistance of STEC strains could be associated with changes in survival to different environmental stresses by characterizing the inactivation kinetics using predictive models. Table 1 shows the antibiotic resistant profile of the strains used in the study. The strains were selected per their antibiotic resistance profiles (high, low and susceptible) and typical STEC virulence factor profiles, Shiga toxin 1 (*stx1*), Shiga toxin 2 (*stx2*), intimin (*eaeA*), and enterohaemolysin (*hlyA*). All strains selected contained *stx1* genes and three strains (High-O26, Sus-O26 and Sus-O103) contained also *stx2* gene.

A recent phylogenetic analysis with whole genome sequence data of O103, O111 and O26 shows H lineages are closer in phylogenetic trees to another similar H serotypes, regardless the O serogroups (Ju et al., 2012). The authors suggested STEC serotypes with the same H antigens

might share common ancestors. In our study, however, strains were chosen based on the antibiotic resistance and O serogroups from the six specific O serogroups declared adulterants in raw beef trimmings by USDA (FSIS, 2011). All of the O26 serogroup strains belonged to the same serotype H11, however, two strains of O103 belonged to H2, and one strain of O103 belonged to H18. Thus the differences seen in the paper are mainly attributable to the antibiotic resistance. However, the authors acknowledge that different H lineages may also have influenced the different survival patterns.

3.1. Effects of LA on STEC inactivation kinetics

The effects of LA on O26 and O103 strains for up to 10 min at room temperature are described in Table 2. Tables 2A and 2B represent the Weibull and log-linear inactivation kinetic parameters for O26 and O103 strains, respectively. As expected, LA treatments were more effective with increasing concentration and exposure time as indicated by greater k (min^{-1}) and lower α values (min) at 5% LA treatment (Table 2). However, no statistical differences ($P>0.05$) were found among kinetic parameters at 3.5 and 5% for O26 and 2.5 and 3.5% for O103 serogroups. Inactivation of 2 and 5 log CFU/ml were observed after 5% LA treatment for 7 and 10 min, respectively. Similar results were obtained by Zhao et al., (2014) where 5.0 and 3.3 log CFU/ml reductions were achieved after 3% LA treatment applied for 10 min for O26 and O103 serogroups inoculated in pure culture, respectively. No statistical differences were found in terms of the inactivation level achieved when the same serotypes were inoculated in beef trims. In the same terms, Fouladkhah et al., (2012) obtained an inactivation level of 0.8 and 1.1 log CFU/ml for O26 and 0.5 and 1.1 log CFU/ml for O103 serogroups after 5% LA treatment at 25 and 55°C for 30 s, respectively. Recently, Dikici et al., (2015) found inactivation levels of 1.8 and 2.7 log CFU/ml for O26 and 2.0 and 2.6 log CFU/ml for O103 after applying 2.5% LA treatment for 3

212 min inoculated in spinach and soy bean sprouts, respectively. The higher reduction levels
 213 achieved in our study are explained by the use of a longer exposure time and/or higher LA
 214 concentration.

215 *D* values for O26 and O103 strains ranged from 2.23-76.6 and 2.4-46.0 min respectively.
 216 However, no statistical differences ($P>0.05$) were found among α and k values at 3.5 and 5%
 217 between O26 and O103 strains. At 2.5% O26 strains were significantly more resistant to LA
 218 treatment than O103 except for the susceptible strain where no statistical differences were found.
 219 This is in agreement with Fouladkhah et al., (2012) and Dikici et al., (2015) where both studies
 220 also did not find significant differences among all the STEC serotypes evaluated including
 221 O157:H7. Log-linear model provided a better fit for survival curves at LA concentrations up to
 222 3.5% that displayed a linear pattern. At 5% most of the curves displayed a nonlinear behavior
 223 with the appearance of a tail (β values lower than 1) and therefore Weibull represented a better
 224 fit.

225 The antibiotic profile resistance did not influence the resistance to LA treatments. No statistical
 226 differences were observed ($P>0.05$) among the three strains of O26 and O103 serogroups with
 227 the exception of the susceptible O26 strain that became more sensitive to LA treatment only at a
 228 low concentration (2.5%). Stopforth et al., (2008) observed a slightly higher thermal resistance
 229 (not statistically different) of antibiotic susceptible *Salmonella* serotypes than the multidrug-
 230 resistant counterparts. The authors speculated that the energy used to regulate the antibiotic
 231 resistance genes may render the multidrug-resistant serotypes more susceptible to different
 232 stresses. On the other hand, Cutter and Rivera-Betancourt (2000), Arthur et al., (2008) and
 233 Fouladkhah et al., (2012) did not find differences in the resistance to different interventions used

in the beef industry (including lactic acid) in *Salmonella* Newport and *Salmonella* Typhimurium multidrug-resistant and susceptible strains.

3.2. Effects of sodium hypochlorite (SH) treatment on inactivation kinetics

Bacterial cells were sharply declined after treating with 50, 40, 30, 20 and 10 ppm free chlorine solutions and no bacterial colonies were detected on plates by 2.5 min (data not shown). Kinetic

parameters after SH treatments for O26 and O103 serogroups are shown in Table 3 (A and B).

No statistical differences ($P>0.05$) were observed among kinetic parameters at 0.2 and 0.5 ppm free chlorine treatments for both serotypes but higher inactivation kinetics were obtained with 1 ppm. The higher SH susceptibility was also observed in terms of log reduction after treatment and 2.5 log CFU/mL inactivation occurred immediately after 1 ppm free chlorine treatment and no cells were detected (>5 log reduction) by 5 min. This is in accordance to Shen et al., (2013) where the authors achieved a 4.5 log reduction on O26 and O103 serogroups after SH treatment at 0.5 ppm for 5 s.

Survival curves showed a more nonlinearity behavior (appearance of shoulders at low concentrations and tails at higher concentrations) after chlorine treatment and therefore Weibull model provided a better fit. No statistical differences were found ($P>0.05$) among kinetic parameters for both serogroups, indicating similar chlorine resistance.

Contrary to the LA results, the antibiotic profile appeared to affect chlorine resistance The susceptible strain for both O26 and O103 serogroups had significantly ($P<0.05$) less resistance to chlorine than the other strains. This may be explained by the different mechanisms of action of both antimicrobials. The acquisition of antibiotic resistance is usually related to a specific target site (horizontal DNA transfer or mutations in housekeeping genes) (Courvalin, 2005).

Bactericides, unlike the antibiotics, exert their effects through non-specific targets (Bower and Daeschel, 1999). For bactericides to be effective, they must be able to penetrate the cell envelope and attain a concentration at the target site high enough to exert their antimicrobial action (Virto et al., 2005). Chlorine is generally considered to be a nonselective oxidant which reacts avidly with a variety of cellular components and affects metabolic processes. Venkobachar et al., (1977) reported that chlorine exposure affected important physical, chemical and biochemical properties of *E. coli* cell membrane affecting the vital functions of bacterium. Virto et al., (2005) pointed out that even more subtle events, such as uncoupling of the electron chain or enzyme inactivation either in the membrane or in the cell interior, are also involved in the bactericidal mechanism of chlorine. On the contrary, the inactivation mechanism of lactic acid is based on the acid dissociation inside the cell that generates protons that acidifies the cytoplasm (IFT, 2006). The more specific target of lactic acid seems not to be affected by the resistance to antibiotics whereas chlorine which has a broader spectrum of action seems to share some of the same mechanisms that affect antibiotic resistance. This is merely a speculation and more research is needed to confirm this hypothesis.

Despite the differences observed between the susceptible and mid- and high-resistant antibiotic strains after SH treatment, in general a strong correlation based on different antibiotic resistance profiles and susceptibility to chlorine treatment was not found. Oscar et al., (2013) did not find statistical differences among several *Salmonella* Typhimurium isolates from broiler chicken carcasses with three different antibiotic profiles (susceptible, resistant to two and six antibiotics) after treatment in chlorinated water (30 ppm). Same results were obtained by Geornaras et al., (2012) where no statistical differences were obtained among *S. Typhimurium* and *S. Newport* susceptible and multi-drug resistant strains after chlorinated water treatment.

3.3. Effects of HT on inactivation kinetics

Table 4 shows the effects of the thermal treatment on O26 and O103 strains (Table 4A and B). As expected, a higher temperature was correlated with faster inactivation (higher k values and lower α values). At 62.5°C, all the STEC strains were reduced by 5 log CFU/ml within 2 min and no bacterial colonies were detected beyond that time regardless of the antibiotic profile. D values ranged from 0.37-2.09 and 0.37-1.71 min for O26 and O103 serogroups, respectively. No statistical differences were observed among both serotypes or antibiotic profile strains within a serotype ($P>0.05$). Vasan et al., (2013) obtained the D values at 54.6-63.6°C for O26 (4.62-0.08 min) and O103 (31.73-0.19 min) finding the O103 serogroup more thermo-tolerant. Bacon et al., (2003), Walsh et al., (2005) and Stopforth et al., (2008) did not find statistical differences among different antibiotic-resistant and susceptible *Salmonella* strains to heat treatment (55-70°C) in buffer and chicken meat. Vasan et al., (2013) did not find statistical differences among the z -values that ranged from 4.10-5.21°C in six STEC serotypes including O26 and O103.

4. CONCLUSION

The aim of this study was to compare the tolerance of antibiotic-resistant versus susceptible O26 and O103 STEC strains to common beef industry interventions namely lactic acid, sodium hypochlorite and heat. In general, Weibull model provided a better fit to the experimental data due to the appearance of nonlinear patterns in the survival curves. A strong correlation could not be found between the antibiotic profile of STEC strains and their resistance to the different stresses. Only the susceptible strains seemed to behave differently, being more resistant to lactic acid and more sensitive to sodium hypochlorite than their antibiotic-resistant counterparts. Different inactivation mechanisms exerted by these biocides could explain those differences.

301 Antibiotic resistance did not affect the thermal tolerance of strains. The role of antibiotic
302 resistance strains in the food chain and processing environments still guarantees more scientific
303 studies that elucidate the real public health implications of their presence. The more extended use
304 of predictive microbiology and risk assessment will aid at reducing the uncertainty level
305 surrounding these complex issues.

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Table 1: List of bacterial strains used in the study.

<i>E. coli</i> strains (Source)	Genotypic and phenotypic profile						Antibiotic resistance*
	O	H	<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>	<i>hlyA</i>	
High-O26 (30-2A-Ca, FSML)	26	11	+	+	-	+	8 (Amp, Cef, Ox, Flo, Cli, Sul, Ti, Til)
High-O103 (10-4aA, FSML)	103	2	+	-	+	+	8 (Amp, Cef, Spc, Ox, Cli, Sul, Ti, Til)
Low-O26 (E25, FSML)	26	11	+	-	+	+	3 (Pen, Cli, Sul)
Low-O103 (E68, FSML)	103	18	+	-	+	+	3 (Cli, Sul, Tet)
Sus-O26 (O26-3, USMARC)	26	11	+	+	+	-	0
Sus-O103 (O103-3, USMARC)	103	2	+	+	+	-	0

*Amp = Ampicillin, Pen = Penicillin, Cef = Ceftiofur, Spc = Spectinomycin, Ox = Oxytetracycline, Cli = Clindamycin, Sul = Sulphadimethoxime, Ti = Tiamulin, Til = Tilmicosin, Tet = Tetracycline; FSML = Food Safety Microbiology Laboratory; USMARC = U.S. Meat Animal Research Center, USDA-ARS, Clay Center, NE.

Table 2: Log-linear and Weibull inactivation kinetic parameters and standard error describing the effects of lactic acid in STEC strains.

2A. Serotype O26.

Lactic acid (%)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
2.5%	0.03±0.01	0.65	67.91±114.48 ^a	0.56	0.04±0.00	0.98	60.21±21.29 ^a	0.97	0.21±0.04	0.86	10.45±0.82 ^{a*}	0.87
3.5%	0.34±0.04	0.91	7.47±1.16 ^b	0.91	0.17±0.02	0.92	14.22±2.64 ^b	0.91	0.47±0.12	0.74	7.03±3.13 ^{ab}	0.67
5%	0.90±0.24	0.72	7.05±0.71 ^b	0.94	1.03±0.18	0.87	5.02±0.84 ^c	0.96	0.92±0.18	0.83	5.67±0.87 ^b	0.95

2B. Serotype O103

Lactic acid (%)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
2.5%	0.10±0.01	0.95	22.99±5.73 ^a	0.94	0.05±0.01	0.88	20.58±3.47 ^a	0.96	0.15±0.04	0.71	16.16±7.80 ^a	0.64
3.5%	0.21±0.02	0.95	10.88±0.62 ^b	0.97	0.12±0.03	0.79	23.86±12.68 ^a	0.77	0.13±0.02	0.89	18.48±5.65 ^a	0.86
5%	0.92±0.26	0.70	6.80±0.33 ^c	0.99	0.94±0.14	0.89	5.05±1.10 ^b	0.93	0.91±0.23	0.74	6.63±0.18 ^b	1.00

¹: Different letters indicate statistical differences among the treatments for the same antibiotic profile. Asterisk indicates statistical difference among the antibiotic profile strains at the same treatment.

Table 3: Log-linear and Weibull inactivation kinetic parameters and standard error describing the effects of sodium hypochlorite treatments in STEC strains.

3A. Serotype O26

Free chlorine (ppm)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
0.2	0.28±0.07	0.70	3.18±2.99 ^a	0.78	0.35±0.05	0.88	3.00±0.79 ^a	0.98	0.61±0.07	0.93	1.60±0.49 ^a	0.98
0.5	0.65±0.04	0.98	3.65±0.70 ^a	0.97	0.61±0.04	0.97	2.52±0.41 ^a	0.99	0.76±0.09	0.91	0.98±0.65 ^{ab*}	0.94
1	1.13±0.05	0.99	1.76±0.41 ^a	0.99	0.95±0.12	0.92	0.68±0.10 ^b	1.00	1.16±0.34	0.68	0.05±0.09 ^{b*}	0.89

3B. Serotype O103

Free chlorine (ppm)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
0.2	0.40±0.05	0.90	2.72±1.09 ^a	0.95	0.36±0.07	0.82	2.13±1.18 ^{ab}	0.93	0.63±0.09	0.90	0.85±0.28 ^{a*}	0.99
0.5	0.62±0.06	0.94	1.83±0.50 ^a	0.98	0.60±0.05	0.96	2.07±0.42 ^a	0.99	0.75±0.15	0.80	0.16±0.10 ^{bc*}	0.98
1	1.20±0.30	0.75	0.30±0.34 ^b	0.92	0.86±0.22	0.73	0.41±0.55 ^b	0.86	1.05±0.34	0.62	0.03±0.04 ^{c*}	0.92

¹: Different letters indicate statistical differences among the treatments for the same antibiotic profile. Asterisk indicates statistical difference among the antibiotic profile strains at the same treatment.

Table 4: Log-linear and Weibull inactivation kinetic parameters and standard error describing the effects of thermal treatments in STEC strains.

4A. Serotype O26

Temperature (°C)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
60	1.10±0.14	0.95	2.29±0.35 ^a	0.98	1.42±0.31	0.87	1.60±0.90 ^a	0.91	1.86±0.76	0.62	2.52±0.26 ^a	0.95
61	3.80±1.38	0.62	1.60±0.20 ^{ab}	0.86	3.78±1.48	0.58	1.33±0.16 ^a	0.90	4.62±0.95	0.85	1.55±0.09 ^b	1.00
62.5	6.10±1.66	0.76	1.18±0.18 ^b	0.95	5.96±1.58	0.77	1.10±0.34 ^a	0.85	5.98±1.38	0.82	0.82±0.44 ^c	0.90

4B. Serotype O103

Temperature (°C)	High				Low				Susceptible			
	Log-linear		Weibull		Log-linear		Weibull		Log-linear		Weibull	
	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²	k (min ⁻¹)	Adj. R ²	α (min) ¹	Adj. R ²
60	1.51±0.52	0.71	2.53±0.28 ^a	0.92	1.55±0.48	0.76	2.42±0.28 ^a	0.93	1.34±0.24	0.91	1.86±0.67 ^a	0.83
61	3.40±1.38	0.50	2.58±0.14 ^a	0.91	3.12±1.60	0.36	2.34±0.12 ^a	0.89	2.73±1.64	0.26	1.76±0.16 ^a	0.85
62.5	5.48±2.34	0.53	1.12±0.56 ^b	0.63	6.11±1.98	0.68	1.26±0.23 ^b	0.91	5.93±2.11	0.63	1.40±0.18 ^a	0.92

¹: Different letters indicate statistical differences among the treatments for the same antibiotic profile. Asterisk indicates statistical difference among the antibiotic profile strains at the same treatment.