

Development of Predictive Models for the Survival of *Campylobacter jejuni* (ATCC 43051) on Cooked Chicken Breast Patties and in Broth as a Function of Temperature

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ABSTRACT

The objective of this study was to model the kinetics of the survival of *Campylobacter jejuni* on cooked chicken breast patties and in broth as a function of temperature. Both patties and broth were inoculated with 10⁶ stationary-phase cells of a single strain of *C. jejuni* (ATCC 43051) and incubated at constant temperatures from 4 to 30°C in 2°C increments under aerobic conditions. In most cases, a three-phase linear model fit the primary survival curves well ($r^2 = 0.97$ to 0.99) at all incubation temperatures regardless of model medium, indicating the presence of a resistant subpopulation of *C. jejuni* that would not be eliminated without thermal processing. Secondary models predicting lag time (LT) and specific death rate (SDR) as functions of temperature were also developed. The Davey and Boltzmann models were identified as appropriate secondary models for LT and SDR, respectively, on the basis of goodness of fit (Boltzmann model, $r^2 = 0.96$; Davey model, $r^2 = 0.93$) and prediction bias and accuracy factor tests. The results obtained indicate that *C. jejuni* can survive well at both refrigeration and ambient temperatures regardless of model medium. Reduced survival of *C. jejuni*, characterized by shorter lag times and faster death rates, was observed both on patties and in broth at ambient temperatures. In addition, the average maximum reduction of *C. jejuni* at 4 to 30°C was 1.5 log units regardless of storage temperature or model medium. These findings suggest that *C. jejuni* found on contaminated poultry products has the potential to survive under conditions that are not permissive for growth and thus could cause foodborne illness if the poultry is not sufficiently cooked.

Campylobacter jejuni causes more cases of foodborne illness each year than any other bacterial pathogen, including *Salmonella*. *C. jejuni* causes between 1,000,000 and 7,000,000 cases of gastroenteritis per year, resulting in 100 to 500 deaths in the United States (7). Although *C. jejuni* is highly susceptible to a wide variety of poultry-processing steps, such as scalding, chilling, and antimicrobial rinses, the organism still manages to survive in birds brought to market (33). It is reported that *C. jejuni* may be present on up to 64% of turkeys and 89% of chickens produced in the United States (9). In addition, a recent survey study reported that the majority (70%) of chicken samples from supermarkets near Washington, D.C., were contaminated with *Campylobacter* (43).

C. jejuni is microaerophilic, growing best in an atmosphere of 5% O₂, 10% CO₂, and 85% N₂, and thermophilic, growing best at 42 to 43°C. Thus, usual food storage conditions are not associated with the growth of *C. jejuni* (1, 34). However, it is important to predict how well *C. jejuni* will survive on chicken at refrigeration and ambient temperatures, because as few as 100 cells can cause illness (31). *C. jejuni* challenge studies have been conducted with poultry (2, 4, 23, 30), red meat (3, 18, 20), pork skin (5), butter (42), and milk (8, 16). These studies have dealt with

the effects of temperature (2–5, 8, 16, 20, 23), pH (8, 14), atmosphere (5, 22, 30), and drying (5, 15) on the survival of *C. jejuni*. The survival of *C. jejuni* during poultry scalding and chilling has also been reported (40) and modeled with the Weibull distribution (41).

Blankenship and Craven (4) reported that *C. jejuni* survived along with the spoilage flora during both air and CO₂ atmosphere storage at 4°C. Koidis and Doyle (22) studied the effects of bisulfite, atmospheric oxygen content, and temperature on the death of *C. jejuni* to define the optimum conditions for the survival of *C. jejuni* and reported that temperature was the most influential factor affecting survival and death. In addition, their results indicated that *C. jejuni* survived best in a medium containing 0.01% sodium bisulfite that was held in an anaerobic environment and maintained at 4°C. On the other hand, Lee et al. (23) demonstrated that *C. jejuni* remained viable at –20 and –70°C, could withstand repeated freeze-thaw cycles, and was able to replicate at 4°C and at ambient temperature. Additionally, Tang and Schraft (36) studied the conditions that enhance the development of *C. jejuni* biofilms. These authors reported that *C. jejuni* counts for biofilms grown at 23°C under aerobic conditions were about 100-fold higher than those for biofilms grown under microaerophilic conditions at 42°C. These two studies showed that *C. jejuni* can grow at ambient temperatures under aerobic conditions, a finding

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that is contrary to the results of other previous studies (3–5, 8, 16, 20).

Although a number of challenge studies (5, 19, 35) have demonstrated the survival of *C. jejuni* on poultry under various storage conditions, these studies were not designed to generate sufficient data to develop models. In addition, it is difficult to consolidate data from the different studies to develop the models because the experimental conditions are not complementary. Furthermore, the survival of *C. jejuni* may be affected by the model medium, but no study has reported the kinetics of the survival of *C. jejuni* on cooked products as well as in unagitated broth that simulates a static medium like ready-to-eat foods in the retail market. Therefore, the objective of this study was to conduct a systematic series of challenge studies with cooked chicken patties and brucella broth to develop predictive models for the survival of *C. jejuni* as a function of temperature.

MATERIALS AND METHODS

Cultures. A strain of *C. jejuni* (ATCC 43051, American Type Culture Collection, Rockville, Md.) was used to develop predictive models for survival. This strain was maintained at -70°C in brucella broth (Difco Laboratories, Sparks, Md.) containing 10% glycerol with 0.16% agar. For each experiment, stock cultures of *C. jejuni* were thawed at room temperature, and then 10 μl of the resuspended stock culture was added to 9 ml of sterile brucella broth. For inoculation, stationary-phase *C. jejuni* cells were obtained by incubating starter cultures in 25-ml Erlenmeyer flasks sealed with a foam plug in a microaerobic jar that contained a CampyPak Plus (Difco), producing 5% O_2 , 10% CO_2 , and 85% N_2 , at 37°C for 23 h at 100 rpm. Viable cell counts for starter cultures at the end of the incubation were between 9.0 and 9.4 log CFU/ml.

Preparation and inoculation of sterile cooked chicken patties and brucella broth. Boneless chicken breast meat was obtained from a local supermarket (Food Lion, Princess Anne, Md.) and was ground twice through a 3/16-in. plate of an electric meat grinder (Oster, Hattiesburg, Miss.). Ten grams of ground chicken was formed into a circular patty. A 1.2-cm² indentation was made in the center of the chicken patty with a dilution tube cap to serve as an inoculation well (26). The chicken patties were cooked by autoclaving at 121°C for 15 min to remove the background microflora. The patties weighed 6 g after autoclaving.

After cooling, the sterile cooked chicken breast patties were transferred to petri dishes under sterile conditions and stored at 4°C in plastic bags to prevent drying (26). Challenge studies were initiated by inoculating 100 μl of stationary-phase *C. jejuni* cells onto the surfaces of the sterile cooked chicken patties as well as into 250-ml Erlenmeyer flasks that contained 50 ml of brucella broth with the use of a sterile repeater pipette for a target population of approximately 8.0 log CFU per patty or 6.0 log CFU per ml. Both inoculated patties and broths were stored at constant temperatures from 4 to 30°C in 2°C increments to investigate and model the survival kinetics of *C. jejuni*.

Enumeration. At selected times after inoculation, depending on the incubation temperature, a cooked chicken breast patty (6 g after autoclaving) was homogenized (Model 400 Stomacher, Seward, London, UK) for 2 min in 94 ml of 0.1% sterilized peptone water. One milliliter of broth medium was diluted in 9 ml of 0.1% sterilized peptone water. Fifty microliters of two dilutions of each

homogenized chicken sample or broth medium was spiral plated (Autoplate 4000, Spiral Biotech Inc., Norwood, Mass.) onto Karmali plates (Oxoid Inc., Nepean, Ontario, Canada) and incubated in a 42°C CO_2 incubator (VWR) under microaerophilic conditions (5% O_2 , 10% CO_2 , and 85% N_2) for 48 h. Colonies on duplicate plates of each sample were counted with an automated colony counter (Q Count, Spiral Biotech). The mean for duplicate plates was plotted at each sampling time to generate the survival curves. Experiments for patties and broth were replicated three and two times, respectively.

pH and a_w measurement. The pHs and water activity values (a_w) of the cooked chicken patties and the brucella broth were measured with an IQ 240 pH meter with a nonglass probe (IQ Scientific Instruments Inc., San Diego, Calif.) and an a_w meter (Aqualab series 3TE, Decagon Devices Inc., Pullman, Wash.), respectively.

Primary modeling. Survival curves of viable cell count (Y , in log CFU per milliliter) versus sampling time (t , in hours) were iteratively fit to a three-phase linear model (Prism, version 3.0, GraphPad Software, San Diego, Calif.) to determine lag time (LT, in hours) and specific death rate (SDR, in log CFU per hour) at each incubation temperature:

$$Y = \begin{cases} \text{IC} & \text{if } t \leq \text{LT} \\ \text{IC} + \left(\frac{\text{FC} - \text{IC}}{A - \text{LT}} \right) (t - \text{LT}) & \text{if } \text{LT} < t < A \\ \text{FC} & \text{if } t \geq A \end{cases} \quad (1)$$

where IC is the initial viable cell count (in log CFU per milliliter), A is the time (in hours) at the start of the tailing phase, and FC is the final cell count (in log CFU per milliliter). SDR and maximum log reduction (MLR) were calculated as

$$\text{SDR} = (\text{FC} - \text{IC}) / (A - \text{LT}) \quad (2)$$

$$\text{MLR} = |\text{FC} - \text{IC}| \quad (3)$$

Secondary modeling. The model of Davey (12) was entered in Prism and used to model LT as a function of temperature (T , in degrees Celsius).

$$\text{LT} = A + (B/T) + (C/T^2) \quad (4)$$

where A , B , and C are regression coefficients without biological meaning. SDR was modeled as a function of temperature (in degrees Celsius) with the Boltzmann sigmoidal equation in Prism:

$$\text{SDR} = \text{SDR}_{\min} + \frac{\text{SDR}_{\max} - \text{SDR}_{\min}}{1 + \exp[(T_{50} - T)/\text{slope}]} \quad (5)$$

where SDR_{\min} is the minimum SDR, SDR_{\max} is the maximum SDR, T_{50} is the temperature at which the SDR is halfway between SDR_{\min} and SDR_{\max} , and slope describes the rate of change of SDR as a function of temperature between SDR_{\min} and SDR_{\max} .

Performance of the models. The goodness of fit of the data to each model was evaluated with the coefficient of determination (r^2) and the standard deviation of the residuals ($S_{y,x}$), which were provided by GraphPad Prism. In addition, the relative error (RE) of each prediction case was calculated by the following equation (13):

$$\text{RE} = \frac{X_p - X_o}{X_p} \quad (6)$$

where X_p is the predicted LT or SDR and X_o is the observed LT or SDR. The median relative error (MRE) was used as the mea-

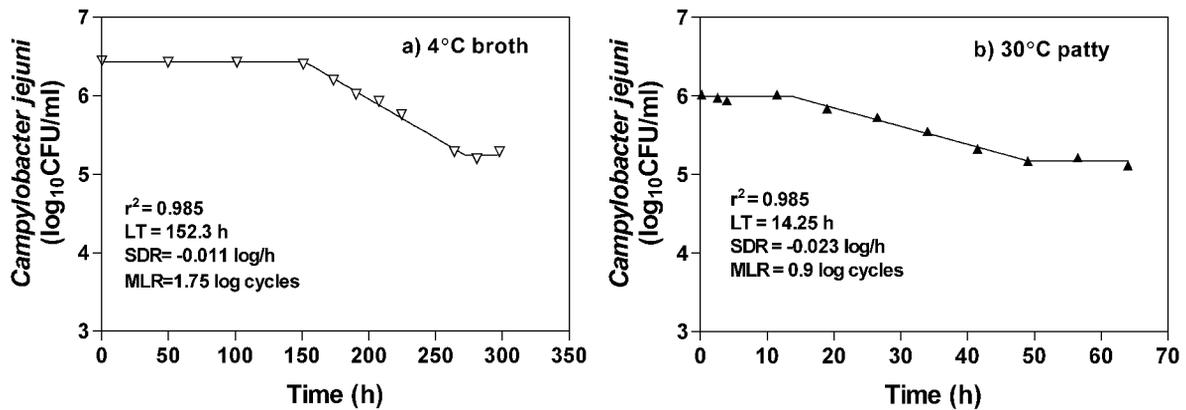


FIGURE 1. Examples of primary model fits to kinetic data for *C. jejuni* collected in broth (a) and on sterile cooked chicken patties (b).

sure of model prediction bias, whereas the mean absolute relative error (MARE) was used as the measure of model prediction accuracy.

Prediction bias and accuracy were also quantified by calculating the bias factor (B_f) and the accuracy factor (A_f) of Ross (32) by the following equations:

$$B_f = 10^{\sum \log(X_p/X_o)/n} \quad (7)$$

$$A_f = 10^{\sum |\log(X_p/X_o)|/n} \quad (8)$$

where n is the number of prediction cases used in the calculation.

Finally, systematic prediction bias was evaluated by visual examination of RE plots and by using Prism to calculate the number of runs, where a run is a set of consecutive residuals either above or below zero on the RE plot.

RESULTS AND DISCUSSION

Primary modeling. For thermal and nonthermal inactivation of vegetative microorganisms, there are four commonly observed types of survival curves in the literature: linear curves, curves with a shoulder, curves with tail-

ing, and sigmoidal curves (21, 37, 39). These survival curves have been modeled with a variety of mathematical formulae (39). Figure 1 shows representative survival curves for *C. jejuni* under the nonthermal inactivation conditions used in the present study. An initial lag phase was followed by a linear decrease in cell population, which varied by rate depending on the temperature, and a bottom plateau of survival, indicating the presence of a resistant subpopulation of *C. jejuni* that would not be eliminated without thermal processing.

As expected, viable cell counts decreased as a function of time in brucella broth (Fig. 1a) and on sterile cooked chicken breast patties (Fig. 1b). A reduction of about 1 log unit was achieved for broth stored at 4°C after 260 h of incubation (Fig. 1a) and for patties at 30°C after 50 h (Fig. 1b), indicating the ability of *C. jejuni* to survive longer at a lower temperature.

Table 1 shows best-fit values and a statistical summary of the primary modeling step. In general, the kinetic data

TABLE 1. Best-fit values (BFV) and statistical summary of primary modeling step^a

T (°C)	Lag time BFV (h)		Specific death rate BFV (log CFU/h)		Goodness of fit				Maximum log reduction (log CFU) ^b		No. of nonthermal inactivation curves	
	Patties	Broth	Patties	Broth	r^2		S_{yx}		Patties	Broth	Patties	Broth
4	149.70	152.30	-0.011	-0.011	0.985	0.996	0.046	0.034	2.17	1.75	2	2
6	44.00	59.17	-0.011	-0.012	0.985	0.994	0.171	0.050	2.13	1.46	2	2
8	51.96	34.07	-0.011	-0.013	0.972	0.996	0.112	0.036	2.07	1.28	2	2
10	29.27	54.53	-0.011	-0.014	0.991	0.983	0.098	0.113	1.87	1.83	3	3
12	36.17	33.23	-0.009	-0.009	0.987	0.981	0.970	0.102	0.92	1.41	3	3
14	32.47	28.68	-0.013	-0.011	0.982	0.992	0.990	0.054	1.30	1.17	3	3
16	31.64	17.21	-0.014	-0.013	0.986	0.989	0.985	0.082	1.08	1.17	3	3
18	19.40	25.40	-0.019	-0.021	0.978	0.995	0.990	0.068	1.62	1.54	3	3
20	18.00	21.22	-0.022	-0.025	0.990	0.988	0.985	0.061	1.81	1.14	3	2
22	10.32	12.93	-0.025	-0.026	0.985	0.995	0.990	0.053	1.53	1.61	2	2
24	5.67	5.56	-0.023	-0.022	0.975	0.972	0.970	0.081	0.86	1.11	2	2
26	12.31	14.34	-0.025	-0.026	0.984	0.978	0.975	0.063	1.29	1.01	2	2
28	10.88	14.32	-0.025	-0.027	0.975	0.992	0.990	0.046	1.64	1.32	2	3
30	14.25	13.68	-0.023	-0.025	0.985	0.992	0.990	0.072	0.90	1.83	2	2
Mean	33.29	34.76	-0.017	-0.018	0.983	0.989	0.086	0.070	1.51	1.40	2.43	2.43

^a r^2 , coefficient of determination; S_{xy} , standard error of the residuals.

^b Log reduction of *C. jejuni* viable cell counts on patties and in broth during storage at each temperature.

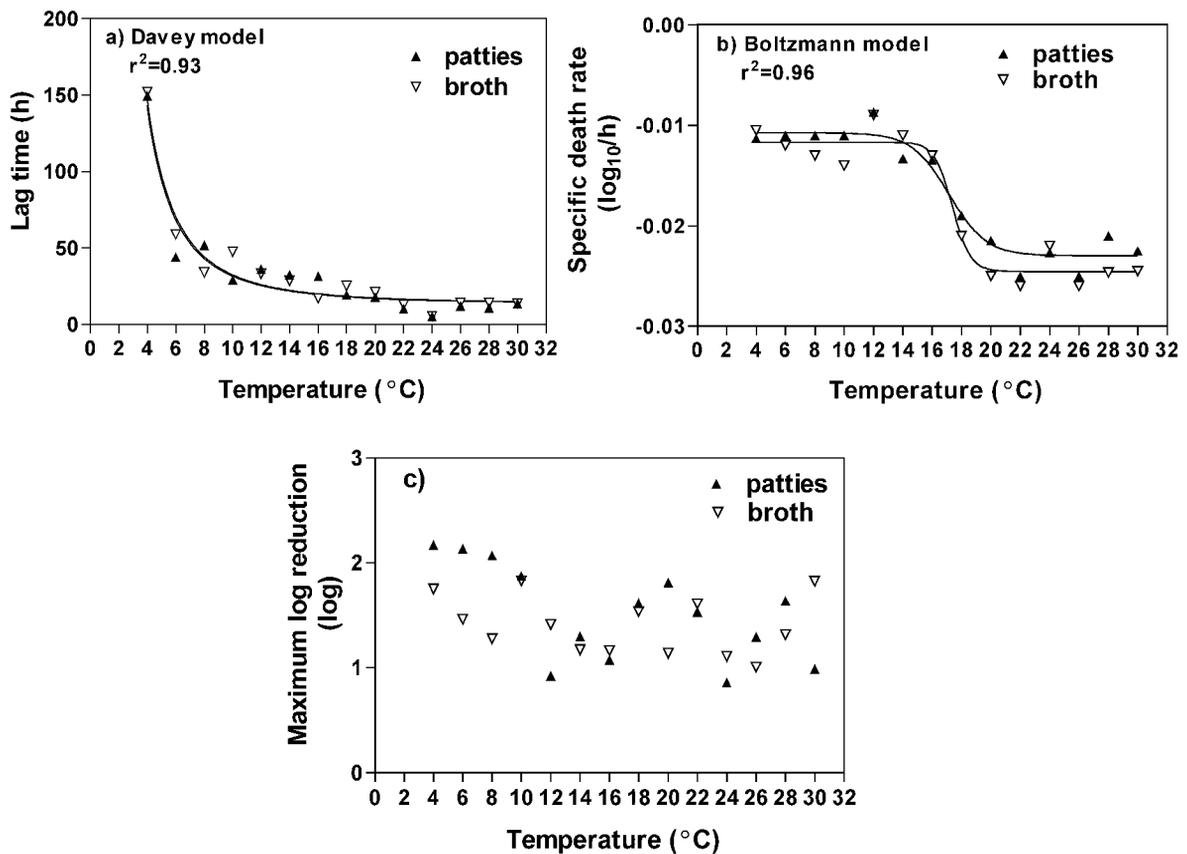


FIGURE 2. Secondary model fits of (a) lag time (LT), (b) specific death rate (SDR), and (c) maximum log reduction (MLR) data as a function of temperature.

for both sterile cooked chicken patties and brucella broth fitted a three-phase linear model well, with a high degree of goodness of fit ($r^2 = 0.97$ to 0.99) and low S_{yx} values at all incubation temperatures (Table 1). In addition, there were no significant differences between the LT or SDR best-fit values for patties and broth according to the two-tailed t test, indicating that *C. jejuni* survival levels on patties and in broth were very similar at all temperatures investigated in the present study. In addition, Table 1 shows the maximum log cycle reduction of *C. jejuni* on patties and in broth at various storage temperatures. The maximum log cycle reduction of *C. jejuni* ranged from 0.86 to 2.17 log units, and the average maximum log cycle reduction of *C. jejuni* on patties or in broth at 4 to 30°C was 1.49 log units. These results indicate that the risk of *C. jejuni* survival under the present nonthermal inactivation conditions was not affected by storage temperature or model medium.

In 1995, Curtis et al. (10) studied the survival of antibiotic-resistant strains of *C. jejuni* in various foods such as chicken, beef, pâté, rice pudding, and mashed potatoes. These investigators also compared their survival data, which were limited to only three temperatures (2, 10, and 20°C), with predictions from a broth model for the survival of *C. jejuni* (Food MicroModel) and concluded that *C. jejuni* survived longer at lower temperatures in all foods and that inactivation was most rapid in pâté, indicating variation in the survival kinetics of *C. jejuni* for different types of food. In addition, *C. jejuni* survives better in cooked beef

products than in raw products (10), a finding that is attributed to the lower pH of raw products (pH 5.6 versus 6.1) (14). On the other hand, Abram and Potter (1) found very similar survival patterns for *C. jejuni* at 6 and 10°C in raw and cooked chicken and beef over a 5-day storage period. However, these investigators also observed that *C. jejuni* survival was better in cooked fish than in raw fish. In the present study, no significant difference between survival patterns for *C. jejuni* in cooked chicken patties and in brucella broth was observed, although there are some differences in their intrinsic properties, such as differences with regard to nutrients, structure (solid versus liquid), pH (6.35 for cooked chicken patties and 7.17 for brucella broth), and a_w (0.987 for cooked chicken patties and 0.998 for brucella broth).

Under the nonthermal inactivation conditions used in the present study, neither model system provided the optimum microaerophilic conditions for inoculated *C. jejuni*, and these conditions were used to simulate conditions in the retail market, where *C. jejuni* could experience a range of atmospheres from aerobic to anaerobic. In the present study, *C. jejuni* was inoculated onto the surfaces of sterile cooked chicken patties, which were incubated in petri dishes enclosed in plastic storage bags. The inoculated *C. jejuni* was absorbed into patties during storage, implying that the inoculated populations of *C. jejuni* might be exposed to both aerobic and anaerobic conditions during the incubation period. In addition, the broth that was inoculated with *C.*

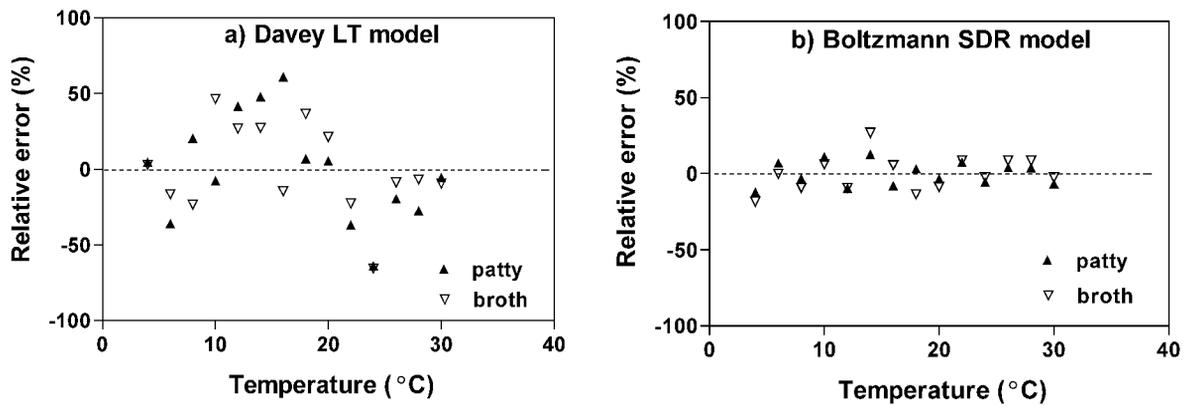


FIGURE 3. Relative error plots of the secondary models for (a) lag time (LT) and (b) specific death rate (SDR) as a function of temperature.

jejuni was not agitated during incubation in the present study, resulting in a gradient of aerobic and anaerobic conditions in the medium. However, the results obtained in the present study indicate that the survival of *C. jejuni* was not significantly affected by model medium. Overall, *C. jejuni* can survive better at lower temperatures. Shorter lag times and faster death rates were observed for patties and for broth stored at ambient temperatures ($>18^{\circ}\text{C}$; Table 1). Some other studies have also indicated that *C. jejuni* survives better at lower temperatures (8, 14, 25).

Secondary modeling. While the kinetics of microbial inactivation in response to thermal processing has been studied and modeled extensively (24, 29, 41), very few studies have involved the modeling of nonthermal inactivation (6, 38). In the present study, secondary models were developed to describe the primary model parameters LT (Fig. 2a) and SDR (Fig. 2b) as functions of temperature. Since the secondary survival model for LT as a function of temperature had a shape very similar to that of the secondary growth model for LT (27), six different growth models for LT, including hyperbola (exp), hyperbola (\wedge^2), hyperbola (\wedge^m) (27), inverse Ratkowsky, and a new lag model (17), were evaluated along with the model of Davey (11) for their ability to predict LT as a function of temperature. Among the models tested, the Davey model was selected as the best-fitting secondary survival model for LT for both patties and broth according to the highest coefficient of determination (>0.93) and the lowest S_{yx} .

For the SDR, we found that the Boltzmann sigmoidal

equation from GraphPad PRISM gave the best fit to data for both cooked chicken patties and broth (Fig. 2b). However, because temperature did not affect the maximum log cycle reduction, we were unable to develop a secondary model for this primary model parameter (Fig. 2c).

Table 2 presents a statistical summary of the secondary modeling step for LT and SDR for cooked chicken patties and broth. The goodness of fit of the model was assessed on the basis of r^2 and S_{yx} . The data used for the development of the secondary models were also used to calculate REs for each prediction case. In turn, the REs of the prediction cases were used to calculate the MRE, a measure of model prediction bias, as well as the MARE, a measure of model prediction accuracy. In addition, the bias factor (B_f) and the accuracy factor (A_f) of Ross (32) were calculated. The bias factor indicates by how much, on average, a model overpredicts ($B_f > 1$) or underpredicts ($B_f < 1$) the observed data. A_f indicates by how much, on average, the prediction differs from the observed data. In equations 5 and 6, a value of 1.0 represents perfect average agreement between the model predictions and observations. However, the value of B_f can also be less than or greater than 1, whereas, the value of A_f is always greater than or equal to 1, since A_f is the absolute value of the logarithm of the ratio. For the Davey LT model, B_f values of 1.08 and 1.06 for patty and broth, respectively, were obtained, indicating that, on average, the model predicted LTs for patties and broth that were, respectively, 8 and 6% longer than those actually observed. This finding indicates that the predicted

TABLE 2. Statistical summary of the secondary modeling step for lag time (LT) and specific death rate (SDR)^a

Model	Medium	Goodness of fit		Prediction bias		Prediction accuracy		Runs test		
		r^2	S_{yx}	MRE	B_f	MARE	A_f	No. of runs	Above	Below
LT	Chicken	0.926	10.73	-0.38	1.08	27.46	1.35	6	7	7
	Broth	0.956	8.414	-7.73	1.06	23.51	1.29	6	6	8
SDR	Chicken	0.966	0.0013	1.17	1.00	5.80	1.06	10	6	8
	Broth	0.951	0.0016	0.49	1.01	7.34	1.08	9	7	7

^a r^2 , coefficient of determination; S_{yx} , standard error of the residuals; MRE, median relative error; B_f , bias factor; MARE, mean absolute relative error; A_f , accuracy factor.

values provide a margin of safety and thus that predictions were fail-safe. However, the average A_f value for both patties and broth was 1.32, demonstrating significant deviation between predicted and observed LT values, a finding that is also supported by the relatively high MARE for the LT models for patties and broth. The MRE and the MARE were -0.38 and 27.46% , respectively, for patties and -7.73 and 23.51% , respectively, for broth (Table 1).

On the other hand, the MREs for the SDR model were close to 0 (1.17% for patties, 0.49% for broth), indicating that this model had low prediction bias. Both the B_f and the A_f values for the SDR model were also found to be close to 1 for patties (1.00 and 1.06 , respectively) and broth (1.01 and 1.08 , respectively), indicating that the SDR model was accurate and showed low prediction bias (Table 2). The accuracy of the SDR model predictions was also assessed on the basis of the MARE. For patties and broth, MAREs were 5.8 and 7.34% , respectively.

As shown in Table 2, goodness-of-fit criteria based on high r^2 values and low S_{yx} and MARE values were better for the Boltzmann SDR model than for the Davey LT model. In addition, the B_f and A_f values for the SDR model were closer to 1 than those for the LT model were. Furthermore, the runs test, which quantifies the distribution of the residuals around 0, was used to evaluate the systematic bias of the model predictions. The model with largest number of runs and thus the most random distribution of its residuals around 0 was found to be the Boltzmann model for SDR (Table 2), but both the LT model and the SDR model showed a lack of systematic prediction bias (Fig. 3). Thus, the fits achieved for the secondary model for LT were not as good as the fits achieved for the secondary model for SDR, a result that was also obtained in another study (17). Overall, the current models provide reliable predictions of both LT and SDR and predict LTs longer than those observed and SDRs similar to those observed, implying that the current models can be regarded as fail-safe. However, the present models were based on the survival of one strain of *C. jejuni* (ATCC 43051); thus, more extensive data for various strains are needed to properly evaluate the potential effect of strain variation on the survival kinetics of *C. jejuni*.

In summary, the survival of *C. jejuni* ATCC 43051 on sterile cooked chicken patties or in brucella broth exhibited three phases: an initial lag phase, a linear death phase, and a stationary survival phase. The average total log cycle reductions of *C. jejuni* on patties and in broth were only 1.51 and 1.40 log cycles, respectively, at all incubation temperatures. The kinetics of death were affected by temperature but not by model medium in the present study. In fact, survival was better at refrigeration temperatures than at ambient temperatures. The remarkable survival potential of *C. jejuni* ATCC 43051, as demonstrated in the present study, may help explain the high percentage of poultry products contaminated with *C. jejuni* in the retail market, as shown in recent survey studies (9, 42), as well as the continuously high rate of isolation of *Campylobacter* from chicken (28). Therefore, *C. jejuni*-contaminated poultry products may cause food poisoning if the poultry is not properly handled

or sufficiently cooked by consumers. The newly developed secondary models for LT and SDR for *C. jejuni* on sterile cooked chicken patties and in brucella broth will be incorporated into the USDA Agricultural Research Service Pathogen Modeling Program, in which they can be easily used to predict the survival kinetics of *C. jejuni* as a function of temperature.

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