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An Approach for Mapping the Number and Distribution of *Salmonella* Contamination on the Poultry Carcass†

T. P. OSCAR*

U.S. Department of Agriculture, Agricultural Research Service, Microbial Food Safety Research Unit and Agricultural Research Service, 1890 Center of Excellence in Poultry Food Safety Research, Room 2111, Center for Food Science and Technology, University of Maryland Eastern Shore, Princess Anne, Maryland 21853, USA

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ABSTRACT

Mapping the number and distribution of *Salmonella* on poultry carcasses will help guide better design of processing procedures to reduce or eliminate this human pathogen from poultry. A selective plating media with multiple antibiotics (xylose-lysine agar medium [XL] containing *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) and the antibiotics chloramphenicol, ampicillin, tetracycline, and streptomycin [XLH-CATS]) and a multiple-antibiotic-resistant strain (ATCC 700408) of *Salmonella* Typhimurium definitive phage type 104 (DT104) were used to develop an enumeration method for mapping the number and distribution of *Salmonella* Typhimurium DT104 on the carcasses of young chickens in the Cornish game hen class. The enumeration method was based on the concept that the time to detection by drop plating on XLH-CATS during incubation of whole chicken parts in buffered peptone water would be inversely related to the initial log number (N_0) of *Salmonella* Typhimurium DT104 on the chicken part. The sampling plan for mapping involved dividing the chicken into 12 parts, which ranged in average size from 36 to 80 g. To develop the enumeration method, whole parts were spot inoculated with 0 to 6 log *Salmonella* Typhimurium DT104, incubated in 300 ml of buffered peptone water, and detected on XLH-CATS by drop plating. An inverse relationship between detection time on XLH-CATS and N_0 was found ($r = -0.984$). The standard curve was similar for the individual chicken parts and therefore, a single standard curve for all 12 chicken parts was developed. The final standard curve, which contained a 95% prediction interval for providing stochastic results for N_0, had high goodness of fit ($r^2 = 0.968$) and was N_0 (log) = 7.78 ± 0.61 − (0.995 × detention time). Ninety-five percent of N_0 were within ±0.61 log of the standard curve. The enumeration method and sampling plan will be used in future studies to map changes in the number and distribution of *Salmonella* on carcasses of young chickens fed the DT104 strain used in standard curve development and subjected to different processing procedures.

Mapping the number and distribution of *Salmonella* contamination on poultry carcasses is a high priority research area for the U.S. Department of Agriculture, Food Safety and Inspection Service, because it will allow better targeting of sampling for *Salmonella* detection, and it will allow better design of processing procedures to reduce or eliminate this human pathogen from poultry carcasses. However, there are no reports in the scientific literature demonstrating how to map the number and distribution of *Salmonella* on poultry carcasses. Therefore, the general objective of this study was to develop an approach for quantitative mapping of *Salmonella* contamination on poultry carcasses.

The approach taken was to divide carcasses of young chickens (28 days of age) in the Cornish game hen class into 12 parts as an initial sampling plan for future mapping studies. Cornish game hens were used because their small size allowed use of less isolation media and made it easier to develop mapping concepts, and because little information is available about the level of *Salmonella* contamination on this class of poultry.

The carcass rinse method coupled with the most-probable-number method is the most common approach used to enumerate *Salmonella* contamination on chicken carcasses. A limitation of the carcass rinse method is that it does not recover all bacteria, including *Salmonella,* from the chicken carcass *(9, 11)* and thus, is less sensitive at detecting *Salmonella* than is the whole-part incubation method *(5, 16).*

Chen et al. *(4)* demonstrated that the drop-plate method is a low-cost and simple direct-plating method for quantifying bacteria in culture media. Blackburn and Davies *(3)* reported that a selective agar medium (i.e., xylose-lysine) supplemented with antibiotics can be used to recover multiple-antibiotic-resistant (MAR) *Salmonella* by direct plating from food-sample incubations. These studies indicate that drop plating onto a selective agar medium with multiple antibiotics might be a cost-effective method for enumerating MAR *Salmonella* in whole-part incubations. Recently, a selective media (xylose-lysine agar medium [XL] containing *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) [HEPES] and the antibiotics chloramphenicol, ampicillin, tetracycline, and streptomycin [XLH-CATS]) was developed for detection and enumeration of a MAR strain of *Salmonella* Typhimurium DT104 in chicken sam-

* Author for correspondence. Tel: 410-651-6062; Fax: 410-651-8498; E-mail: Thomas.Oscar@ars.usda.gov.

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TABLE 1. *Weight and experimental design for inoculation of chicken parts with* Salmonella *Typhimurium DT104: dilution of culture used for inoculation in each trial*

Chicken part		Chicken part wt (g)		Trial(s)					
Code	Name	Mean	SD	1, 7	2, 8	3	4, 9, 10	5, 11	6, 12
a	Wing, left	42.9	2.3	−7[a]	−2	−3	−4	−5	−6
b	Wing, right	42.4	7.8	−7	−2	−3	−4	−5	−6
c	Breast, front left	56.1	6.8	−6	−7	−2	−3	−4	−5
d	Breast, front right	56.9	9.2	−6	−7	−2	−3	−4	−5
e	Breast, back left	36.1	5.5	−5	−6	−7	−2	−3	−4
f	Breast, back right	40.3	3.7	−5	−6	−7	−2	−3	−4
g	Back, rib	70.8	12.6	−4	−5	−6	−7	−2	−3
h	Back, sacrum	80.2	15.4	−4	−5	−6	−7	−2	−3
i	Thigh, left	51.5	8.1	−3	−4	−5	−6	−7	−2
j	Thigh, right	49.5	4.7	−3	−4	−5	−6	−7	−2
k	Drumstick, left	43.6	4.5	−2	−3	−4	−5	−6	−7
l	Drumstick, right	47.4	3.0	−2	−3	−4	−5	−6	−7

[a] Dilutions of −7, −6, −5, −4, −3, and −2 correspond to initial log doses of 0.5 to 1, 1.5 to 2, 2.5 to 3, 3.5 to 4, 4.5 to 5, and 5.5 to 6, respectively.

ples, with other microorganisms *(13, 14).* Therefore, the specific objective of this study was to develop a standard curve for enumerating *Salmonella* Typhimurium DT104 on Cornish game hen carcass parts by drop plating on XLH-CATS, for use in future mapping studies with MAR strains of *Salmonella.*

MATERIALS AND METHODS

Organism. A MAR strain (ATCC 700408, American Type Culture Collection, Manassas, Va.) of *Salmonella* Typhimurium DT104 was used to develop the enumeration method. Stock cultures were maintained at −70°C in brain heart infusion (Difco, Becton Dickinson, Sparks, Md.) broth containing 15% (vol/vol) glycerol (Sigma, St. Louis, Mo.).

Chicken preparation. Cornish game hen carcasses were purchased from local retail outlets and stored frozen until they were thawed in a domestic-type refrigerator. Thawed carcasses were processed into 12 parts (Table 1), which were placed into individual 500-ml polycarbonate jars (Fisher Scientific, Hampton, N.H.). The carcasses ($n = 12$) had an average weight of 618 g (range of 581 to 705 g).

Chicken part inoculation and incubation. Five microliters of stock culture was added to 5 ml of brain heart infusion broth in a 25-ml Erlenmeyer flask and sealed with a foam plug, which was followed by incubation at 30°C for 23 h and 150 rpm, before serial dilution to 10^{-7} in buffered peptone water (BPW; Difco, Becton Dickinson). Chicken parts in polycarbonate jars were spot inoculated with 2 μl (trials 7 to 12) or 5 μl (trials 1 to 6) of the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, or 10^{-7} dilution of the 23-h culture for an initial dose (N_0) of 5.5 to 6 log, 4.5 to 5 log, 3.5 to 4 log, 2.5 to 3 log, 1.5 to 2 log, or 0.5 to 1 log, respectively. The amount of *Salmonella* inoculated was systematically rotated among the different types of chicken parts and trials, per the schedule shown in Table 1.

Inoculated parts were held at room temperature for 15 min, which was followed by addition of 300 ml of BPW (40°C) and incubation for 24 h at 40°C, with gentle shaking at 100 rpm. At 1, 2, 3, 4, 5, 6, 7, 8, and 24 h of incubation, 2 μl of BPW was removed and drop plated onto XL agar medium (Difco, Becton Dickinson) containing 25 mM HEPES and 25 μg/ml of each of the following antibiotics: chloramphenicol, ampicillin, tetracycline, and streptomycin: XLH-CATS. All media supplements were from Sigma.

Enumeration method. The drop plates were incubated for a standard time (24 h) and temperature (38°C), and the image on the drop plate was captured using an automated digital colony counter (Protocol, Microbiology International, Frederick, Md.). The Paintbrush software program (version 5.0, Microsoft Corporation, Redmond, Wash.) was used to convert the captured image to a monochrome image. The pixels in each drop of the monochrome image were counted using Image J 1.34s software (Wayne Rasband, National Institutes of Health, Bethesda, Md.; available at: http://rsb.info.nih.gov/ij/). A graph for all 12 chicken parts within a trial was constructed by plotting the pixels per drop (Y) as a function of sampling time (X). The resulting density curves ($n = 12$) were fit (version 4.0, Prism software, GraphPad, Inc., San Diego, Calif.) to the following sigmoid equation:

$$Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10^{(X_{50}-X)})$$

where bottom was fixed at zero pixels per drop, top was constrained to being shared among the 12 curves, and X_{50} was the time (in hours) when the curve reached 50% of maximum, which was the detention time (DT; in hours).

A standard curve for enumeration was generated by graphing the initial log number (N_0) of *Salmonella* Typhimurium DT104 as a function of DT and fitting (Prism software) the data to a linear equation:

$$N_0 = a + (b \times \text{DT})$$

where a was the y intercept, and b was the slope. The curve fit also generated a 95% prediction interval (PI) that provided stochastic projections for N_0.

RESULTS

The threshold value of the drop-plate assay for detection of *Salmonella* Typhimurium DT104 was estimated by drop plating different dilutions of the inoculation culture onto XLH-CATS (Fig. 1). Detectable growth on XLH-CATS after 24 h at 38°C was observed at an inoculation culture concentration of 10^3/ml and above. At an inocula-

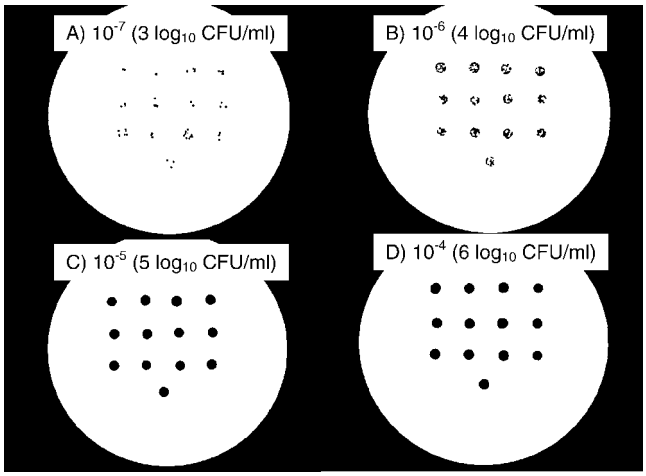

FIGURE 1. *Monochrome images of drop plates from different dilutions of the inoculation culture of* Salmonella *Typhimurium DT104. Thirteen drops were plated per dilution.*

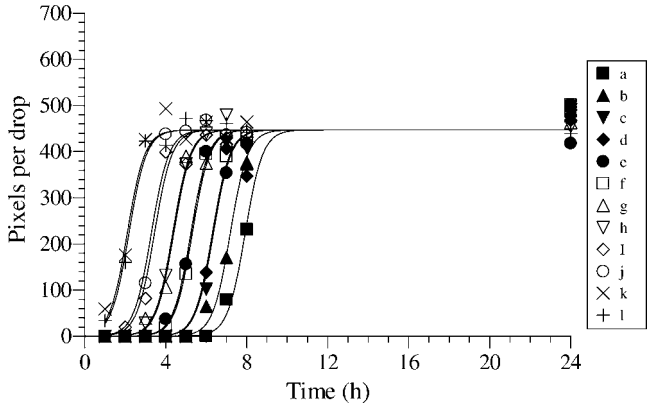

FIGURE 3. *Curve fits for determination of detection time (DT) in trial 7. Symbols are for the different chicken parts as follows: a, left wing; b, right wing; c, front left breast; d, front right breast; e, back left breast; f, back right breast; g, back, rib; h, back, sacrum; i, left thigh; j, right thigh; k, left drumstick; and l, right drumstick.*

tion culture concentration of 10^5/ml and above, the growth within the drop was confluent, whereas at 10^3 and 10^4/ml, growth within the drop was not confluent.

When chicken parts were inoculated with *Salmonella* Typhimurium DT104 and then incubated in BPW for 24 h at 40°C, the growth observed on drop plates went from no growth at 0 h to confluent growth at 24 h (Fig. 2). The time at which growth on drop plates became confluent was inversely related to N_0. For example, growth for $N_0 = 10^{5.5}$ was confluent by 3 h of incubation (Fig. 2C), whereas growth for $N_0 = 10^{2.5}$ was not confluent until 6 h of incubation (Fig. 2F).

When the amount of growth within each drop was graphed as a function of time, the data fit well ($r^2 = 0.966 \pm 0.038$ [mean $\pm$ standard deviation], $n = 144$) to a sigmoid equation (Fig. 3). The time for the curve to reach 50% of maximum or the time for the drop to reach 50% of confluent growth was taken as DT. Based on results in Figure 1, the density curves in Figure 3 can be interpreted as follows. The bottom phase of the density curves occurred when the level of *Salmonella* Typhimurium DT104 in BPW of the whole-part incubation was below 10^3/ml, the top phase of the density curves occurred when the level of *Salmonella* Typhimurium DT104 in BPW of the whole-part incubation was 10^5/ml or above, the exponential phase of the density curves occurred when the level of *Salmonella* Typhimurium DT104 in the whole-part incubation was between 10^3 and 10^5/ml, and the DT or time when the density curve was 50% of the way between bottom and top occurred when the level of *Salmonella* Typhimurium DT104 in the whole part incubation was about 10^4/ml.

When N_0 (log) was graphed as a function of DT, a linear standard curve was obtained (Fig. 4). The standard

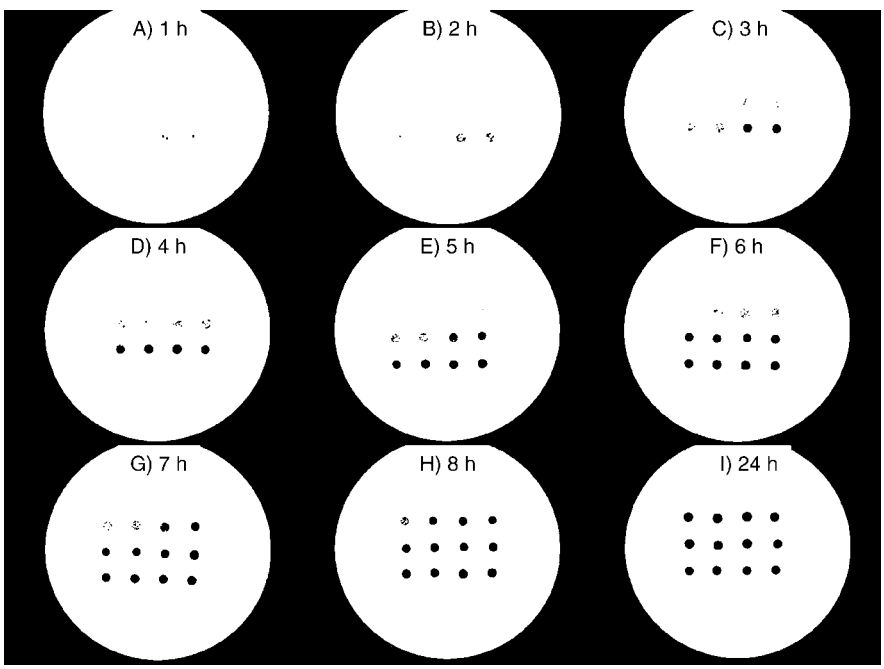

FIGURE 2. *Monochrome images of drop plates from trial 7. Each drop corresponds to a different chicken part, and each pair of drops in a row corresponds to a different inoculated dose (0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 log) of* Salmonella *Typhimurium DT104. Doses increase across rows and down columns, i.e., the pair of drops in row 1, columns 1 and 2, corresponds to 0.5 log, and the pair of drops in row 3, columns 3 and 4, corresponds to 5.5 log.*

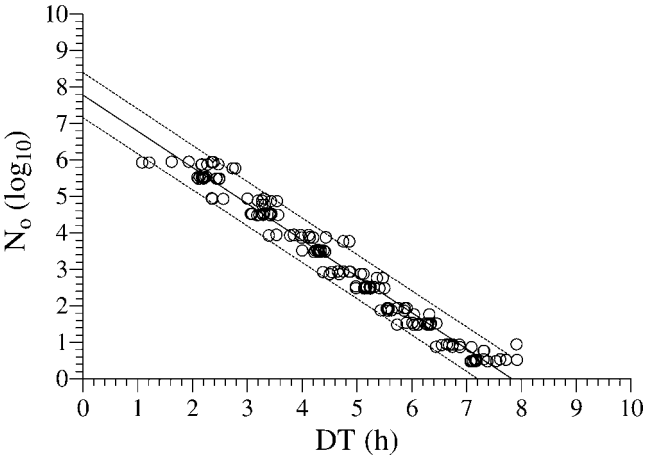

FIGURE 4. *Standard curve and its 95% prediction interval for stochastic projection of* Salmonella *Typhimurium DT104 (initial number,* N_0*) on chicken parts as a function of detection time (DT).*

curve, which included a 95% PI and had high goodness of fit (R^2 = 0.968), was as follows: $N_0 = 7.78 \pm 0.61 - (0.995 \times \text{DT})$.

The 95% PI indicated that 95% of the values of N_0 were within ±0.61 log of the standard curve. The correlation (r) between N_0 and DT was −0.984.

The method provides stochastic results for N_0 by using the 95% PI to calculate lower and upper bounds of the standard curve (Table 2). The range of the assay is 0 to 6 log per chicken part and, therefore, a calculated N_0 above 6 log is reported as >6 log. The results in Table 2 are a subset of the results presented in Figure 4.

The y intercept and slope of the standard curve was similar among individual chicken parts with coefficients of variation of 3.2% for the y-intercept and 5.1% for the slope (Table 3). The 95% PI had a higher coefficient of variation of 19.2% among individual chicken parts. The average 95% PI for individual parts was ±0.90 log (range of ±0.62 to 1.21 log) as compared with ±0.61 log for the combined data (Fig. 4). The average R^2 (0.972) and r (−0.986) values for the standard curves for individual parts were similar to those for the final standard curve (R^2 = 0.968 and r = −0.984) for all chicken parts combined.

DISCUSSION

Salmonella Typhimurium DT104 was used for standard curve development in this study because it has a phenotype that can be followed and enumerated in the presence of other microorganisms *(13, 14).* The low prevalence of *Salmonella* Typhimurium DT104 on chickens *(1)* is an advantage for standard curve development because interference from indigenous *Salmonella* Typhimurium DT104 is a low probability and, in fact, was not observed (i.e., outliers) in the present study. However, the low prevalence of *Salmonella* Typhimurium DT104 on chickens is a disadvantage in mapping studies using naturally contaminated chickens, as a large number of samples would need to be analyzed to develop an accurate carcass map. Therefore, we plan on feeding *Salmonella* Typhimurium DT104 to chickens and then mapping its distribution on Cornish game hen carcasses, using the standard curve developed in this study. The success of future mapping studies with young chickens fed *Salmonella* Typhimurium DT104 is expected because Fedorka-Cray et al. *(7)* demonstrated that young chickens can be colonized with *Salmonella* Typhimurium DT104 after oral gavages.

Enumeration of bacteria, including *Salmonella,* by detection time during incubation of food samples is not new. In fact, Gibson *(8)* used a commercial impedance system (Bactometer) to enumerate MAR (rifampin- and nalidixic acid–resistant) strains of *Salmonella* Thompson, Stanley, and Infantis by DT in culture medium and pork slurries. Although commercial impedance systems are available that allow automated collection of DT data for liquid food samples, such as carcass rinse samples *(6),* these systems are not configured, nor are they validated for incubation of solid food samples, such as bone-in chicken parts that were used in this study.

TABLE 2. *Selected examples of stochastic results from the standard curve and its 95% prediction interval (PI): log number (*N_0*) of* Salmonella *Typhimurium DT104 inoculated onto chicken parts*

Trial	Chicken part		DT (h)[a]	Predicted N_0 (log)			Inoculated N_0 (log)[b]
	Code	Name		Lower 95% PI	Best fit	Upper 95% PI	
1	a	Wing, left	7.32	0.00	0.41	1.02	0.76
2	b	Wing, right	2.17	5.09	5.70	>6	5.86
3	c	Breast, front left	2.18	5.08	5.69	>6	5.87
4	d	Breast, front right	3.31	3.92	4.53	5.14	4.93
5	e	Breast, back left	2.36	4.90	5.51	>6	4.94
6	f	Breast, back right	3.40	3.83	4.44	5.05	3.92
7	g	Back, rib	4.40	2.80	3.41	4.02	3.51
8	h	Back, sacrum	5.15	2.03	2.64	3.25	2.51
9	i	Thigh, left	6.12	1.03	1.64	2.25	1.47
10	j	Thigh, right	6.35	0.80	1.41	2.02	1.48
11	k	Drumstick, left	6.13	1.02	1.63	2.24	1.48
12	l	Drumstick, right	7.62	0.00	0.10	0.71	0.53

[a] DT, detection time.

[b] Based on the volume and concentration of culture inoculated as determined by direct plating of the inoculation culture.

TABLE 3. *Standard curve and its 95% prediction interval (PI) as affected by the type of chicken part*

Chicken part		Standard curve				
Code	Name	*y* Intercept	95% PI	Slope	R^2	*r*
a	Wing, left	7.72	0.98	−0.976	0.968	−0.984
b	Wing, right	7.98	0.62	−1.036	0.986	−0.993
c	Breast, front left	7.69	0.64	−0.969	0.985	−0.993
d	Breast, front right	8.15	0.91	−1.062	0.972	−0.986
e	Breast, back left	7.86	0.78	−1.004	0.975	−0.988
f	Breast, back right	7.82	0.97	−1.025	0.962	−0.981
g	Back, rib	7.73	1.06	−0.993	0.966	−0.983
h	Back, sacrum	7.76	0.83	−0.982	0.974	−0.987
i	Thigh, left	7.40	1.02	−0.901	0.965	−0.982
j	Thigh, right	7.50	0.78	−0.958	0.979	−0.989
k	Drumstick, left	8.18	0.94	−1.071	0.973	−0.986
l	Drumstick, right	8.19	1.21	−1.065	0.953	−0.976
Mean		7.83	0.90	−1.003	0.972	−0.986
SD		0.25	0.17	0.051	0.009	0.005
Coefficient of variation		3.2%	19.2%	5.1%	1.0%	0.5%
Minimum		7.40	0.62	−1.071	0.953	−0.993
Maximum		8.19	1.21	−0.901	0.986	−0.976

In the study of Gibson *(8),* the linear correlation in broth between N_0 and DT was −0.959 and in pork slurry was −0.935. In comparison, a linear correlation of −0.984 was obtained in current study between N_0 and DT for whole chicken parts by the drop-plate method. The negative correlation sign indicates an inverse relationship between N_0 and DT. Also, in the study of Gibson *(8),* 95% of N_0 in broth were within ±1 log of the standard curve, whereas 95% of N_0 for pork slurry were within ±1.65 log of the standard curve. In the current study, 95% of N_0 for whole chicken parts were within ±0.61 log of the standard curve. These results indicate that DT is variable among samples with the same N_0, and that deviation of N_0 from the standard curve was less in the current study, which used a drop-plate method for DT, than it was in the study of Gibson *(8),* which used an impedance method for DT.

There are a number of reports of native microflora altering the growth of *Salmonella* during incubation of food samples *(2, 15, 17).* For example, Beckers et al. *(2)* reported that final density of a nalidixic acid–resistant strain of *Salmonella* Typhimurium in BPW, with competitive microflora, ranged from 10^3 to 10^7/ml. Variations in numbers and types of native microflora among chickens (i.e., trials) might help explain the variation of DT within a N_0 observed in the present study. Rather than ignore this biological variation, a standard curve was developed that included a 95% PI that provided stochastic projections for N_0. By definition, the 95% PI should contain 95% of all future DT determined using the same method.

It has been demonstrated that a single 1-min rinse of a chicken carcass only recovers a small percentage of native bacteria *(11),* and that recovery is improved by incubating the whole carcass for 24 h *(5, 16),* which was done in the present study with whole carcass parts. Electron microscopy reveals that during poultry processing, a liquid film forms on the surface of the chicken carcass and becomes contaminated with bacteria from process waters *(18),* and during immersion in communal baths, the skin swells and entraps bacteria in channels and crevices that form *(10, 19, 20).* Continued detection of bacteria, including *Salmonella,* on repeated rinsing of the same carcass likely reflects continued recovery from the liquid film in skin crevices and channels. The impact of entrapped bacteria on DT in the current study was not evaluated. Nonetheless, use of the whole-part incubation method rather than the carcass rinse method in future mapping studies will facilitate the detection and enumeration of *Salmonella* entrapped in carcass skin and will result in more accurate carcass maps.

Sampling times to generate suitable DT curves (i.e., pixels per drop versus time) depend on the assay range, temperature of incubation, volume of incubation medium (i.e., BPW), and volume of sample that is drop plated onto XLH-CATS. In the current study, an incubation temperature of 40°C was used because it is the optimal growth temperature for *Salmonella* Typhimurium DT104 on chicken meat with native microflora *(13, 14),* and an incubation volume of 300 ml was used because it was the minimum volume that provided adequate coverage of the chicken parts, which ranged in average weight from 36 to 80 g. Sampling times at hourly intervals from 1 to 8 h and at 24 h were found to generate suitable DT curves for an assay range of 10^0 to 10^6 *Salmonella* Typhimurium DT104, an incubation volume of 300 ml, and a drop-plate assay volume of 2 μl. Effects of changes in the assay parameters (i.e., assay range, incubation volume, and drop volume) on the standard curve and its 95% PI were not examined. Nonetheless, the standard curve had high goodness of fit (R^2 = 0.968) and a 95% PI (i.e., ±0.61 log) that was less than the 95% PI for enumeration of MAR *Salmonella* by DT, using an impedance method *(8).*

Future studies are planned with Cornish game hens obtained from commercial plants and at retail to use the enumeration method and sampling plan developed in this study to map the distribution of MAR strains of *Salmonella* Ty-

phimurium DT104 and other MAR strains of *Salmonella* that are resistant to the four antibiotics (i.e., chloramphenicol, ampicillin, tetracycline, and streptomycin) used in the drop-plate agar medium (i.e., XLH-CATS). The method developed in this study can be incorporated into the traditional culture method for isolation of *Salmonella* by performing the drop-plate assay during the first 24 h of preenrichment in BPW and followed by isolation of *Salmonella,* using traditional selective enrichment and selective plating steps. If the growth kinetics and DT in BPW of strains detected and enumerated on naturally contaminated chickens differ significantly from those of the strain of *Salmonella* Typhimurium DT104 used to develop the standard curve in this study, then new standard curves would be developed and enumeration data corrected for those strains. A new standard curve may also be needed to properly enumerate MAR *Salmonella* on retail Cornish game hens, which are sold frozen, as freezing was previously found to increase both lag time and DT in conductance studies performed in meat and saline extracts *(12).* Additional research is needed to determine the effect of freezing on the standard curve and its 95% PI.

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REFERENCES

1. Akkina, J. E., A. T. Hougue, R. J. Angulo, R. Johnson, K. E. Petersen, P. K. Saini, P. J. Fedorka-Cray, and W. E. Schlosser. 1999. Epidemiologic aspects, control, and importance of multiple-drug resistant *Salmonella typhimurium* DT104 in the United States. *J. Am. Vet. Med. Assoc.* 214:790–798.
2. Beckers, H. J., J. V. D. Heide, U. Fenigsen-Narucka, and R. Peters. 1987. Fate of salmonellas and competing flora in meat sample enrichments in buffered peptone water and in Muller-Kauffmann's tetrathionate medium. *J. Appl. Bacteriol.* 62:97–104.
3. Blackburn, C. W., and A. R. Davies. 1994. Development of antibiotic-resistant strains for the enumeration of food borne pathogenic bacteria in stored foods. *Int. J. Food Microbiol.* 24:125–136.
4. Chen, C., G. W. Nace, and P. L. Irwin. 2003. A 6 × 6 drop-plate method for simultaneous colony counting and MPN enumeration of *Campylobacter jejuni, Listeria monocytogenes,* and *Escherichia coli. J. Microbiol. Methods* 55:475–479.
5. Cox, N. A., and L. C. Blankenship. 1975. Comparison of rinse sampling methods for detection of salmonellae on eviscerated broiler carcasses. *J. Food Sci.* 40:1333–1334.
6. Edmiston, A. L., and S. M. Russell. 1999. Evaluation of a conductance method for enumerating *Escherichia coli* on chicken, pork, fish, beef, and milk. *J. Food Prot.* 62:1260–1265.
7. Fedorka-Cray, P. J., S. R. Ladely, J. S. Bailey, and N. J. Stern. 2001. Colonization of broiler chicks by *Salmonella* Typhimurium definitive phage type 104. *J. Food Prot.* 64:1698–1704.
8. Gibson, A. M. 1988. Use of impedance measurements to estimate numbers of antibiotic resistant *Salmonella* strains. *Lett. Appl. Microbiol.* 6:89–92.
9. Izat, A. L., W. Yamaguchi, S. Kaniawati, J. P. McGinnis, S. G. Raymond, R. E. Hierholzer, and J. M. Kopek. 1991. Use of consecutive carcass rinses and a most probable number procedure to estimate *Salmonellae* contamination of inoculated broilers. *Poultry Sci.* 70: 1448–1451.
10. Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Lett. Appl. Microbiol.* 22:280–282.
11. Lillard, H. S. 1988. Comparison of sampling methods and implications for bacterial decontamination of poultry carcasses by rinsing. *J. Food Prot.* 51:405–408.
12. Mackey, B. M., and C. M. Derrick. 1984. Conductance measurements of the lag phase of injured *Salmonella typhimurium. J. Appl. Bacteriol.* 57:299–308.
13. Oscar, T. P. 2006. Validation of a tertiary model for predicting variation of *Salmonella* Typhimurium DT104 (ATCC 700408) growth from a low initial density on ground chicken breast meat with a competitive microflora. *J. Food Prot.* 69:2048–2057.
14. Oscar, T. P. 2007. Predictive model for growth of *Salmonella* Typhimurium DT104 from low and high initial density on ground chicken with a natural microflora. *Food Microbiol.* 24:640–651.
15. Rhodes, P., L. B. Quesnel, and P. Collard. 1985. Growth kinetics of mixed culture in *Salmonella* enrichment media. *J. Appl. Bacteriol.* 59:231–237.
16. Simmons, M., D. L. Fletcher, M. E. Berrang, and J. A. Cason. 2003. Comparison of sampling methods for the detection of *Salmonella* on whole broiler carcasses purchased from retail outlets. *J. Food Prot.* 66:1768–1770.
17. Stecchini, M. L., L. Ferraro, and G. Caserio. 1988. Dynamics of *Salmonella* pre-enrichment in buffered peptone water. *Microbiol. Aliments Nutr.* 6:367–371.
18. Thomas, C. J., and T. A. McMeekin. 1980. Contamination of broiler carcass skin during commercial processing procedures: an electron microscopic study. *Appl. Environ. Microbiol.* 40:133–144.
19. Thomas, C. J., and T. A. McMeekin. 1981. Spoilage of chicken skin at 2°C: electron microscopic study. *Appl. Environ. Microbiol.* 41: 492–503.
20. Thomas, C. J., and T. A. McMeekin. 1982. Effect of water immersion on the microtopography of the skin of chicken carcasses. *J. Sci. Food Agric.* 33:549–554.