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Qualitative Map of *Salmonella* Contamination on Young Chicken Carcasses†

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ABSTRACT

Salmonella contamination of poultry is a global public health problem. The objective of this study was to map the distribution of *Salmonella* on the young chicken carcass, to improve poultry inspection and food safety. Young chickens ($n = 70$) in the Cornish game hen class were obtained at retail over a 3-year period. Carcasses were aseptically sectioned into 12 parts, and then *Salmonella* was isolated from whole-part incubations by conventional culture methods. Isolates were characterized for serotype and antibiotic resistance, and by pulsed-field gel electrophoresis (PFGE). *Salmonella* incidence was 21.5% (181 of 840) for parts and 57.1% (40 of 70) for carcasses. The number of contaminated parts per carcass ranged from 0 to 12, with a mean of 4.5 among contaminated carcasses. Chi-square analysis indicated that *Salmonella* incidence differed ($P < 0.05$) among parts, with rib back (38.6%) and sacral back (34.3%) being the most contaminated. Among the 40 contaminated carcasses, there were 37 different patterns of contamination among parts. Of the 33 carcasses with more than one contaminated part, 12.1% contained two serotypes, 33.3% contained two or more antibiotic resistance profiles, and 100% contained two or more PFGE patterns. The most common serotype was Typhimurium (94.5%), and most (97.2%) isolates were resistant to multiple antibiotics. These results indicated a diverse pattern of *Salmonella* contamination among carcasses and that multiple subtypes of *Salmonella* were often present on contaminated carcasses. Thus, whole-carcass incubation succeeded by characterization of multiple isolates per carcass is needed to properly assess and manage this risk to public health.

Maps are important tools used in everyday life. For example, maps are used to travel from one location to another. Maps are also used by military organizations to plan adversarial actions. An important component of a military map is accurate knowledge of the precise location and strength of opposing forces. By analogy, maps of pathogen contamination on food would allow better detection and removal of these risks to public health. However, there are currently no maps of the distribution of pathogens on food, such as animal carcasses, fresh produce, or seafood.

A method for mapping the incidence and number of antibiotic resistant strains of *Salmonella* on the chicken carcass was recently developed by Oscar *(23)*. The method involves dividing the carcass into 12 parts and then using a standard curve to enumerate *Salmonella* as a function of detection time during whole-part incubations. Detection time is determined by drop plating on agar media with multiple antibiotics. A limitation of this method of Oscar is that it is specific for *Salmonella* that are resistant to chloramphenicol, ampicillin, tetracycline, and streptomycin. Thus, information on the antibiotic resistance of resident *Salmonella* is needed before this method can be used for development of a quantitative map.

Most studies indicate that carcasses of chickens and other classes of poultry are contaminated with a diverse population of *Salmonella (13, 16, 18, 35)*. For example, Parveen et al. *(26)* isolated *Salmonella* from whole-carcass incubations before and after chilling in a commercial broiler chicken processing plant, and reported the presence of 13 different serotypes and more than 12 antibiotic resistance profiles. Current studies with these isolates *(25)* indicate a diverse population of pulsed-field gel electrophoresis (PFGE) patterns.

Typically, only one isolate of *Salmonella* is characterized per whole-carcass sample. Consequently, there is a lack of information on the potential diversity of *Salmonella* subtypes on individual carcasses. Therefore, the current study was undertaken (i) to develop a qualitative map of *Salmonella* contamination on the young chicken carcass for the purpose of improving poultry inspection and process control; (ii) to determine the antibiotic resistance of resident *Salmonella* for the purpose of developing a quantitative map in a future study; and (iii) to characterize the diversity of *Salmonella* subtypes within individual carcasses for the

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purpose of developing a map more detailed of *Salmonella* contamination on the young chicken carcass for improving poultry inspection and food safety.

MATERIALS AND METHODS

Chicken carcass preparation. Cornish game hen carcasses ($n = 70$) from the same commercial processing plant and brand were obtained at retail over a 3-year period. The carcasses were thawed at 4°C and then aseptically subdivided into 12 parts, according to the method of Oscar *(23)*: A, left wing; B, right wing; C, left front breast; D, right front breast; E, left back breast; F, right back breast; G, rib back; H, sacral back; I, left thigh; J, right thigh; K, left drumstick; and L, right drumstick. Parts were weighed and placed in 500-ml polycarbonate jars with screw-cap lids for *Salmonella* isolation.

Isolation procedure. All microbiological media were from the same source (Difco, Becton Dickinson, Sparks, MD). Presumptive isolates of *Salmonella* were obtained from whole-part incubations by standard culture methods *(26)*. In brief, 300 ml of sterile buffered peptone water was added to the jar, and then the carcass part was incubated at 37°C and 80 opm for 24 h. Next, 0.1 ml of sterile buffered peptone water was transferred to a tube containing 10 ml of Rappaport-Vassiliadis broth, and then incubated at 42°C and 60 opm for 24 h. An aliquot (10 μl) of Rappaport-Vassiliadis broth was then streaked onto a xylose lysine Tergitol 4 agar plate that was then incubated at 37°C for 24 h. A single black colony (i.e., presumptive isolate of *Salmonella*), if present, was selected at random from the xylose lysine Tergitol 4 plate and inoculated into 5 ml of brain heart infusion broth, and then incubated at 40°C and 150 opm for 24 h. A portion (0.1 ml) of the isolate culture was then transferred to a freezer vial containing 0.9 ml of brain heart infusion broth with 15% (vol/vol) glycerol and then stored at −70°C.

Serotyping. Isolates were serotyped at a *Salmonella* Reference Center (University of Pennsylvania, Kennett Square) by standard methods using reagents prepared in accordance with World Health Organization guidelines.

Antibiotic resistance. Antibiotic resistance was determined at a *Salmonella* Reference Center by an automated antimicrobial susceptibility system (Sensititre, Trek Diagnostic Systems, Westlake, OH), according to the manufacturer's instructions. Antibiotic resistance results were interpreted according to established testing standards and interpretive criteria *(5, 6)*. Resistance was determined for 13 antibiotics (MIC range): amikacin (0.5 to 32 μg/ml), amoxicillin–clavulanic acid (0.5 to 16 μg/ml [amoxicillin]; 1 to 32 μg/ml [clavulanic acid]), ampicillin (1 to 32 μg/ml), cefoxitin (0.5 to 32 μg/ml), ceftriaxone (0.5 to 64 μg/ml), chloramphenicol (2 to 32 μg/ml), ciprofloxacin (0.015 to 4 μg/ml), gentamicin (0.25 to 16 μg/ml), kanamycin (8 to 64 μg/ml), nalidixic acid (0.5 to 32 μg/ml), sulfisoxazole (16 to 512 μg/ml), tetracycline (4 to 32 μg/ml), and trimethoprim-sulfamethoxazole (0.12 to 4 μg/ml [trimethoprim]; 2.38 to 76 μg/ml [sulfamethoxazole]).

PFGE. PFGE was performed according to the method of Ribot et al. *(27)*. Agarose-embedded bacterial genomic DNA was digested with the restriction enzyme *Xba*I (Roche Diagnostics, Indianapolis, IN), according to the manufacturer's instructions. DNA fragments were separated by PFGE in a 1% agarose gel (Bio-Rad, Hercules, CA) by using the CHEF DR-III electrophoresis apparatus. The resulting gel was stained with ethidium bromide, photographed, and analyzed with BioNumerics software (Applied Maths, Sint-Martens Latem, Belgium). Patterns from the same carcass or part were compared with the Dice coefficient of similarity, and only isolates displaying 100% similarity were considered to have the same PFGE pattern.

Statistical analysis. A 2 by 12 contingency table and chi-square test were used to determine whether there was a global difference in *Salmonella* incidence among carcass parts, whereas a 2 by 2 contingency table and Fisher's exact test were used to compare *Salmonella* incidence among paired samples. Statistical tests were performed with version 5.0 of the Prism software program (GraphPad Software Inc., San Diego, CA).

RESULTS

Chi-square analysis indicated that *Salmonella* incidence differed ($P < 0.05$) among carcass parts. *Salmonella* incidence was highest for rib back and sacral back, and in part, this resulted from these parts being larger than the other parts (Table 1). However, *Salmonella* incidence was higher ($P < 0.05$) for chicken wings than it was for chicken thighs, even though chicken wings weighed less than chicken thighs did. There were no differences ($P > 0.05$) in *Salmonella* incidence among the same chicken parts from the left and right sides of the carcasses, except that *Salmonella* incidence was higher ($P < 0.05$) on the right drumsticks than on the left drumsticks. Overall, 181 (21.5%) of 840 of the chicken parts were contaminated with *Salmonella*.

Table 2 shows the random pattern of distribution of the number of contaminated parts per carcass as a function of the date of *Salmonella* isolation. Forty (57.1%) of the 70 carcasses contained at least one part that was positive for *Salmonella*. The number of *Salmonella*-positive parts per contaminated carcass ($n = 40$) ranged from 1 to 12, with an average of 4.5 among the contaminated carcasses.

Thirty-three carcasses were contaminated on two or more parts. Among the 40 contaminated carcasses, there were 37 different patterns of *Salmonella* contamination among parts (Table 2). Of the 33 carcasses with more than one contaminated part, 12.1% (4 of 33) contained two serotypes, 33.3% (11 of 33) contained two or more antibiotic resistance profiles, and 100% (33 of 33) contained two or more PFGE patterns.

The most common *Salmonella* serotype was Typhimurium (94.5%) and most isolates (97.2%) were resistant to three or more antibiotics (Table 3). Occurrence of PFGE patterns from the same carcass with 100% similarity was a rare event involving only 19 of the 181 isolates (Table 2). Figure 1 shows an example of the diversity of PFGE patterns observed among *Salmonella* isolates from the same carcass.

For a small set of samples, the outside (skin) and inside (bone-muscle) portions of the rib back and sacral back were incubated separately. For the rib back, the mean weight of the skin portion was 5.2 g, and the mean weight of the bone-muscle portion was 62.9 g. Of the six rib backs examined, none of the six skin portions were contaminated with *Salmonella,* whereas five of six bone-muscle portions were contaminated with *Salmonella*. For sacral back, the mean weight of the skin portion was 11.3 g, and the mean weight

TABLE 1. *Confirmed isolates of* Salmonella *from young chicken carcasses: incidence as a function of carcass part*

Part code	Part	Carcass part wt (g)		Positive	Total	Incidence (%)
		Mean	SD			
A	Wing, left	40.7	4.3	19	70	27.1
B	Wing, right	41.2	10.7	17	70	24.3
C	Breast, left front	62.2	18.3	20	70	28.6
D	Breast, right front	59.7	15.3	18	70	25.7
E	Breast, left back	35.2	15.8	5	70	7.1
F	Breast, right back	36.8	17.6	6	70	8.6
G	Back, rib	66.8	14.6	27	70	38.6
H	Back, sacral	77.4	17.1	24	70	34.3
I	Thigh, left	47.6	14.2	12	70	17.1
J	Thigh, right	50.0	15.1	9	70	12.9
K	Drumstick, left	44.6	11.6	7	70	10.0
L	Drumstick, right	48.4	13.2	17	70	24.3
A to L	Carcass	611	50	181	840	21.5

of the bone-muscle portion was 72.9 g. Of the five sacral backs examined, one of five skin portions was contaminated with *Salmonella,* whereas one of five bone-muscle portions was contaminated with *Salmonella*.

For a small set of carcass part samples (Table 4), more than one isolate of *Salmonella* was characterized for serotype and antibiotic resistance and by PFGE. Results indicated that 0% contained more than one serotype, 20% contained more than one antibiotic resistance profile, and 90% contained more than one PFGE pattern.

DISCUSSION

The current chicken carcass testing program of the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) uses the whole-carcass rinse sampling method for detection of *Salmonella (34)*. In this method, an aliquot (30 ml) from a single whole-carcass rinse (100 ml) of a chicken carcass is used for *Salmonella* detection by PCR and conventional culture methods. However, Lillard *(12)* demonstrated that a single whole-carcass rinse is not sufficient to detect *Salmonella* on the carcass. Cox and Blankenship *(8)* reported an incidence of 46% when *Salmonella* was determined by incubating the whole carcass in isolation medium, whereas *Salmonella* incidence was only 3.8% when it was determined by the whole-carcass rinse method. Likewise, Simmons et al. *(29)* reported a *Salmonella* incidence of 38% for the whole-carcass incubation method versus a *Salmonella* incidence of only 13% for the whole-carcass rinse method. Despite these results, the whole-carcass rinse method continues to be the method of choice for determining *Salmonella* incidence on the chicken carcass *(2)*.

Simmons et al. *(29)* concluded that the whole-carcass incubation method was more sensitive than was the whole-carcass rinse method when small numbers of *Salmonella* are expected, suggesting and perhaps (rightly so) that it is equivalent to the whole-carcass rinse method when high numbers of *Salmonella* are present. However, most studies in which the numbers of *Salmonella* on the chicken carcass have been determined report small numbers of *Salmonella*. For example, Surkiewicz et al. *(30)* reported that at the exit of the chill tank the percentages of carcasses with <1, 1 to 10, 30 to 300, and >300 cells of *Salmonella* were 79, 16, 1, and 4%, respectively. More recently, Brichta-Harhay et al. *(3)* reported that *Salmonella* levels on chicken carcasses at postchill were very low (i.e., 0.05 $\pm$ 0.005 CFU/ml of rinse fluid). They concluded that current method (i.e., whole-carcass rinse) would have a low chance of detecting such low levels of *Salmonella*. Thus, they concur with the earlier finding of Cox and Blankenship *(8)* that the whole-carcass incubation method is the better method for detecting *Salmonella* on the chicken carcass.

Considering the aforementioned studies, it was decided in this study that the whole-carcass incubation method rather than the whole-carcass rinse method would produce a more accurate map of *Salmonella* contamination on the young chicken carcass. It was believed that whole-carcass incubation, in this case as whole carcass parts, would be the better method for detecting all forms of *Salmonella* associated with the carcass including (i) unattached *Salmonella* in the water layer on the surface of the carcass *(11, 14)*; (ii) *Salmonella* attached to the skin surface *(15)*; (iii) *Salmonella* attached to muscle fascia *(28, 31)*; (iv) *Salmonella* entrapped in skin crevices *(32)*; (v) *Salmonella* trapped between muscle fibers *(1, 33)*; (vi) *Salmonella* trapped in feather follicles *(10)*; and (vii) invasive *Salmonella* present in the residual blood in the deep tissues of the carcass. Furthermore, bacteria that are attached to

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[a] For definitions of abbreviations for carcass parts, see Table 1.

[b] An, amikacin; Ax, amoxicillin–clavulanic acid; Am, ampicillin; Ce, cefoxitin; Cef, ceftriaxone; Cm, chloramphenicol; Cip, ciprofloxacin; G, gentamicin; K, kanamycin; N, nalidixic acid; Su, sulfisoxazole; Te, tetracycline; Tr, trimethoprim-sulfamethoxazole.

[c] R, antibiotic resistant. Blank cells indicate antibiotic susceptibility and <100% similarity of PFGE patterns.

TABLE 2. *Subtyping of* Salmonella *isolated from young chicken carcasses*

Date	Part[a]	Serotype	Antibiotic resistance[b,c]													PFGE similarity (%)
			An	Ax	Am	Ce	Cef	Cm	Cip	G	K	N	Su	Te	Tr	
17-Apr-06	G	Thompson														
25-Apr-06	L	Kentucky		R	R	R							R	R		
22-May-06	C	Typhimurium		R	R	R							R	R		
30-May-06	A, B, C, D, E, F, G, H, L	Typhimurium		R	R	R							R	R		
5-Jun-06	B, C, D, E, F, G, H, I, L	Typhimurium		R	R	R							R	R		
12-Jun-06	A, G, H, I, J, L	Typhimurium		R	R	R							R	R		
	B, C	Typhimurium		R	R	R							R	R		100
19-Jun-06	C	Typhimurium		R	R	R							R	R		
17-Jul-06	A, B, C, D, E, F, G, H, I, J, K, L	Typhimurium		R	R	R					R		R	R		
31-Jul-06	A, B, C, H, J, K	Typhimurium			R			R					R	R		
	E, G, L	Typhimurium		R	R	R							R	R		
18-Sep-06	A	Typhimurium			R			R					R	R		
25-Sep-06	C, G, L, D	Typhimurium		R	R	R							R	R		
16-Oct-06	A	Typhimurium														
	C	Kentucky		R	R	R								R		
	G	Typhimurium		R	R	R					R		R	R		
6-Nov-06	A, B, C, D, G, L, H	Typhimurium		R	R	R							R	R		
13-Nov-06	A, B, D, G, H, J, K	Typhimurium		R	R	R							R	R		
	C	Typhimurium		R	R	R	R						R	R		
27-Nov-06	G, I	Typhimurium		R	R	R							R	R		
4-Dec-06	A, B	Typhimurium		R	R	R							R	R		
4-Dec-06	A, G, L	Typhimurium		R	R	R							R	R		
	B, H	Typhimurium		R	R	R							R	R		100
8-Jan-07	A, C, D, K	Typhimurium		R	R	R					R		R	R		
	B, L	Typhimurium		R	R	R					R		R	R		100
	G, H	Typhimurium		R	R	R					R		R	R		100
22-Jan-07	C, H, I	Typhimurium		R	R	R							R	R		
5-Feb-07	A, B, D, G, I	Typhimurium		R	R	R							R	R		
20-Feb-07	B, D, G, H	Typhimurium		R	R	R							R	R		
26-Feb-07	G	Typhimurium		R	R	R					R		R	R		
5-Mar-07	A, B, C, E, G, K, L	Typhimurium		R	R	R							R	R		
	D, F, H	Typhimurium		R	R	R							R	R		100
12-Mar-07	A, J	Typhimurium		R	R	R							R	R		100
	D, G, H	Typhimurium		R	R	R							R	R		
26-Mar-07	D, H	Typhimurium		R	R	R							R	R		
	G	Typhimurium		R	R	R					R		R	R		
2-Apr-07	C, H	Typhimurium		R	R	R							R	R		
	G	Typhimurium		R	R	R					R		R	R		
9-Apr-07	B, C, D, G	Glostrup								R			R	R		
	L	Typhimurium		R	R	R							R	R		
16-Apr-07	I, K, L	Typhimurium		R	R	R							R	R		
23-Apr-07	G, L	Typhimurium		R	R	R							R	R		
30-Apr-07	B, C, D, G, H, L	Typhimurium		R	R	R							R	R		
	I, J	Typhimurium		R	R	R							R	R		100
28-Jan-08	F, G	Typhimurium		R	R	R							R	R		
14-Feb-08	A, B, C, D, F, G, H, K, L	Typhimurium		R	R	R							R	R		
	I, J	Typhimurium		R	R	R							R	R		100
28-Feb-08	C, G, H	Typhimurium		R	R	R							R	R		
	D	Typhimurium		R	R								R	R		
5-Mar-08	A, B, C, G, H, I, L	Typhimurium		R	R	R							R	R		
2-Apr-08	A	Typhimurium		R	R	R	R				R		R	R		
	H	Typhimurium		R	R	R							R	R		
9-Apr-08	A, H	Typhimurium		R	R	R							R	R		
20-Apr-08	D, H	Typhimurium		R	R	R							R	R		
	I	Enteritidis														
3-Jun-08	J	Enteritidis														
14-Jul-08	A, D	Typhimurium		R	R	R							R	R		100
	H, I	Typhimurium		R	R	R							R	R		
20-Sep-08	H	Enteritidis														
	J	Typhimurium		R	R	R							R	R		

TABLE 3. *Summary of serotypes and antibiotic resistance of* Salmonella *isolated from young chicken carcasses*

Antibiotic resistance[a]	*Salmonella* serotype: Thompson	Kentucky	Typhimurium	Glostrup	Enteritidis	Total	%
None	1		1		3	5	2.8
Ax, Am, Ce, Su, Te		1	136			137	75.7
Ax, Am, Ce, K, Su, Te			24			24	13.3
Am, Cm, Su, Te			7			7	3.9
Ax, Am, Ce, Te		1				1	0.6
Ax, Am, Ce, Cef, Su, Te			1			1	0.6
G, Su, Te				4		4	2.2
Ax, Am, Su, Te			1			1	0.6
Ax, Am, Ce, Cef, K, Su, Te			1			1	0.6
Total	1	2	171	4	3	181	
%	0.6	1.1	94.5	2.2	1.7		

[a] For definitions of abbreviations for antibiotics, see Table 2, footnote *b*.

surfaces and growing as biofilms are known to release daughter cells that can migrate to other locations *(7)*. Thus, during prolonged incubation (i.e., 24 h) of chicken parts, it was believed that firmly attached and trapped cells of *Salmonella* would produce daughter cells that would migrate into the preenrichment broth, grow to high numbers, and be detected, thus resulting in an accurate map of *Salmonella* contamination of the chicken carcass.

Comparison of *Salmonella* incidence among chicken parts in the present study was confounded by differences in size of carcass parts. It has been previously demonstrated that size of the sample affects *Salmonella* incidence *(30),* and that this effect is nonlinear *(22)* and therefore not amenable to correction by covariate analysis. Because of trapped and potentially internalized *Salmonella,* it was concluded that the weight of chicken parts rather than their surface area was the critical factor to consider when interpreting the results.

An objective of this research was to assess the distribution of *Salmonella* among the parts and then in future studies to dissect the parts into component parts (i.e., skin, bone, muscle, and fat) to map more precisely the location of the pathogen. In fact, in the current study, dissection of the rib back into outside (skin) and inside (bone-muscle) portions revealed that the *Salmonella* contamination was mainly associated with the inside portion. A possible explanation is that the inside part of the rib back contained the inner shell of the carcass that was in contact with the crop, which has been identified as an important source of *Salmonella* contamination of the chicken carcass *(9)*. During the feed-withdrawal period just before slaughter, chickens will typically eat litter that could contain *Salmonella*-contaminated feces from themselves or other birds in the flock. During evisceration, some of this ingested litter can leak from the crop and into the cavity of the carcass and contaminate the rib back as the crop is being removed. Although proof of this mechanism of rib back contamination awaits further research, this result, which was obtained with a small number of samples, suggests and confirms previous research that feed withdrawal and crop removal are potential areas where greater process control could be exercised to reduce *Salmonella* contamination of the chicken carcass.

In the current study, it was possible to make statistical comparisons of *Salmonella* incidence among paired carcass parts from the left and right sides of the carcass, without the confounding effect of part weight. To our surprise, there was one notable difference in these comparisons, namely, the right drumsticks had a higher *Salmonella* incidence than the left drumsticks had. A possible explanation for this result is that during evisceration, the carcasses move from left to right, and as the intestines are removed they are

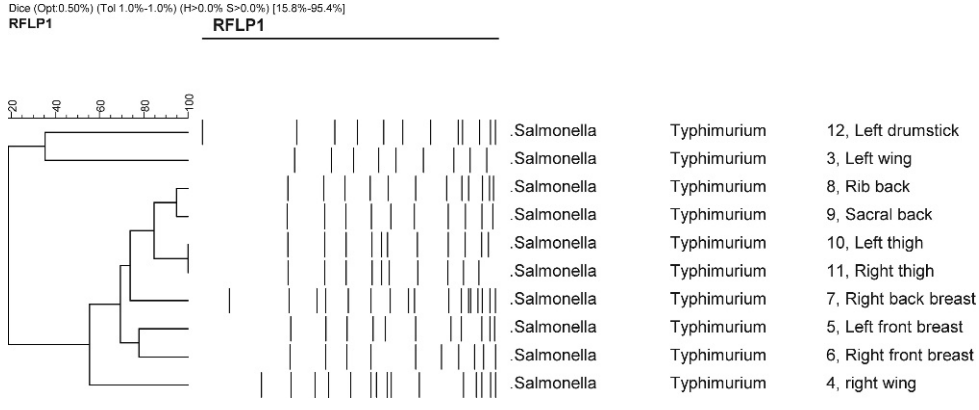

FIGURE 1. *Dendogram of PFGE patterns of* Salmonella *isolates from the same young chicken carcass.*

TABLE 4. *Subtyping of isolates of* Salmonella *from the same whole-part incubation*[a]

Date	Part	Isolate	Serotype	Antibiotic resistance[b]												PFGE similarity (%)
				An	Ax	Am	Ce	Cef	Cm	Cip	G	K	N	Su	Te	Tr	
2-Apr-08	A	a	Typhimurium		R	R	R	R				R		R	R		
	A	b, c, d, e, f	Typhimurium		R	R	R					R		R	R		
	H	a, b	Typhimurium		R	R	R							R	R		100
20-Apr-08	D	a, b, c, d	Typhimurium		R	R	R							R	R		
	H	a, b, c, d	Typhimurium		R	R	R							R	R		
	I	a, b, c, d	Enteritidis														
3-Jun-08	J	a, b, c, d, e	Enteritidis														
14-Jul-08	A	a	Typhimurium		R	R	R							R	R		
	A	b	Typhimurium		R	R								R	R		
	H	a	Typhimurium		R	R	R							R	R		
	H	b	Typhimurium		R	R								R	R		
	I	a, b	Typhimurium		R	R	R							R	R		
20-Sep-08	J	a, b, c	Typhimurium		R	R	R							R	R		

[a] For definitions of abbreviations for carcass parts, see Table 1; for antibiotics, see Table 2, footnote *b*.
[b] R, antibiotic resistant. Blank cells indicate antibiotic susceptibility and <100% similarity of PFGE patterns.

pulled up and laid over the right side of the carcass for proper presentation for carcass inspection, which would increase the probability of intestinal contents leaking onto this part, and this thus could explain its higher rate of *Salmonella* contamination versus the left drumsticks. Future studies are needed to confirm this explanation. However, if true, these results would identify intestinal tract removal as an area where greater process control could be exercised to reduce *Salmonella* contamination of the chicken carcass.

Although the wings weighed less than the thighs did, they had a higher *Salmonella* incidence in the present study. A possible explanation for this result is that during processing, the carcass is hung upside down and washed from the back end to the front end. Thus, *Salmonella* contamination from all the other parts of the carcass is washed toward the wings, where it can be retained. Further research is needed to examine this possibility. These studies would include mapping *Salmonella* contamination on the carcass before and after the inside-outside carcass washer. In addition, these studies would include mapping *Salmonella* contamination within the different parts (drumette, wingette, and tip) of the wing to more precisely identify the location of this pathogen for removal and risk reduction.

An unexpected finding in this study was that most of the isolates of *Salmonella* were resistant to multiple antibiotics. In fact, 163 of the 181 isolates of *Salmonella* used to develop the qualitative map were resistant to the same four antibiotics: amoxicillin, ampicillin, cefoxitin, and tetracycline. Thus, it should be possible in a future study to modify the drop plate media and method of Oscar *(23)* and develop a quantitative map of *Salmonella* contamination for Cornish game hens from the commercial processing plant that served as the source of carcasses for this study. Clearly, there is also a need to develop carcass maps for different commercial processing plants to assess how process variations affect the distribution of *Salmonella* contamination on the chicken carcass.

One of the goals of this research is to improve poultry inspection. The results of this mapping study indicate that *Salmonella* contamination is randomly distributed on individual chicken carcasses but at the population level, there are ''hot spots,'' or areas of the carcass where *Salmonella* contamination is more frequent, thus presenting opportunities to enhance process control and reduce this risk to public health. Results also indicate that multiple subtypes (i.e., serotype, antibiotic resistance, and PFGE patterns) are present on individual carcasses. Thus, to better assess and manage this risk to public health, *Salmonella* incidence measurements should be based on whole-carcass incubations as parts rather than as a single whole-carcass rinse and, multiple isolates of *Salmonella* should be characterized from each carcass instead of none or one.

Capita et al. *(4)* also found multiple subtypes of *Salmonella* in a single sample from a chicken carcass and concluded that it is best to analyze multiple isolates from each carcass. This is important, because accurate knowledge of the serotype, antibiotic resistance, and PFGE patterns of the *Salmonella* that contaminate chicken carcasses are important for assessing its potential risk to public health, as *Salmonella* differs widely in its ability to cause human illness *(21)*. In other words, a higher incidence of *Salmonella* contamination does not necessarily translate into a higher risk to public health if the contaminating serovars are not highly pathogenic in humans. In fact, some serovars (e.g., Kentucky) have less ability to grow on chicken meat; thus, this poses less of a risk to public health than other subtypes *(17, 19, 20, 24)*.

Because of its small size, the Cornish game hen is a good starting point for establishing methods and proving the value of carcass mapping for food safety *(23)*. However, there is no reason why the whole-part incubation method for mapping *Salmonella* contamination on the Cornish game hen carcass cannot be applied to the larger carcasses of broilers, roasters, and turkeys. The only additional costs would be larger incubation vessels, greater volumes of preenrichment broth in the first step of the isolation procedure, and additional incubator space.

Use of other sampling methods (whole-carcass rinse, swabbing, sponging) that are not capable of detecting all forms of *Salmonella* on the poultry carcass will result in inaccurate maps, which in turn will result in an inaccurate assessment and management of this risk to public health. Thus, it is important that mapping studies not be carried out with these methods. The bottom line is that it is better to have a smaller amount of accurate data for a carcass map based on the whole-carcass incubation as parts method than to have a larger amount of inaccurate data from other sampling methods that do not detect all forms of *Salmonella* on the poultry carcass.

In conclusion, results of this study demonstrate, for the first time, the high value carcass mapping has for improving poultry inspection and food safety. The results indicate that carrying out whole-carcass incubation with parts, and then subtyping multiple isolates per carcass, is needed to properly assess and manage this risk to public health. However, this approach is more labor-intensive than is the current approach used in the FSIS monitoring program, and thus, it might not be practical to implement in the field. Nonetheless, carcass mapping as a research tool has potential for identification of critical control points where greater process control can be exercised to reduce the level of pathogens on the chicken carcass. Future research needs in poultry carcass mapping with *Salmonella* and other pathogens include but are not limited to (i) development of quantitative maps for risk assessment; (ii) development of maps for carcass parts; (iii) development of maps for multiple commercial plants; (iv) development of maps for other classes of poultry; and (v) development of maps at different steps in poultry processing.

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