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Original Article

Prevalence and Distribution of *Salmonella* in Organic and Conventional Broiler Poultry Farms

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Abstract

The objective of this cross-sectional study was to compare the prevalence of *Salmonella* and antimicrobial-resistant *Salmonella*, as well as investigate the distribution of this pathogen in organic and conventional broiler poultry farms. Fecal ($n = 420$), feed ($n = 140$), and drinking water ($n = 140$) samples were collected from birds at 3 and 8 weeks of age for 2-flock cycles. One house was sampled per farm at three organic and four conventional broiler farms from the same company in North Carolina. All samples were analyzed for the presence of *Salmonella* using selective enrichment techniques. Further phenotypic (antimicrobial susceptibility) and genotypic (pulsed-field gel electrophoresis [PFGE]) testing were performed. *Salmonella* prevalences in fecal samples were 5.6% (10/180) and 38.8% (93/240) from organic and conventional farms, respectively. From feed, 5.0% (3/60) and 27.5% (22/80) of the samples were positive for *Salmonella* from organic and conventional farms, respectively. None of the water samples were positive for *Salmonella*. Seventy isolates were characterized by antimicrobial susceptibility and PFGE types. The two most common resistance phenotypes were single resistance to streptomycin (36.2% [25/58]: conventional; 25% [3/12] organic), and multidrug resistance to six antimicrobial agents: ampicillin-streptomycin-amoxicillin/clavulanic acid-cephalothin-ceftiofur-cefoxitin (AmStAxChCfFx; 39.7%: conventional only). Genotypic analysis using PFGE showed clonality among isolates within and between the two types of farms. The results of our study suggest that within this poultry company, the prevalence of fecal *Salmonella* was lower in certified-organic birds than in conventionally raised birds, and the prevalence of antimicrobial-resistant *Salmonella* was also higher in conventionally raised birds than in certified-organic birds.

Introduction

According to the Centers for Disease Control and Prevention (CDC), the incidence of *Salmonella* (i.e., 16.2 cases per 100,000 population/year) was the least improved of all foodborne pathogens in terms of achieving national health objective targets for Healthy People 2010 (CDC, 2008). Poultry remains an important vehicle of *Salmonella* transmission to humans, occurring mainly via contaminated meat (Kimura *et al*., 2004; Marcus *et al*., 2007). According to the U.S. Department of Agriculture (USDA)–Economic Research Service, poultry is the fastest growing meat product in the U.S. organic market with a market size estimated to be around $46 million and annual growth estimated to be 33% through 2008 (USDA, 2008). The number of USDA-certified organic broiler birds increased from 2 million in 2000 to over 10 million in 2005 (USDA, 2006). This is due in part to consumer concerns over the way conventional poultry is reared as well as their perception that organic foods are healthier and safer for consumption. Further, many scientists point to antimicrobial use in animal agriculture (e.g., poultry) as the driving force behind development and dissemination of antimicrobial-resistant *Salmonella* (Ford *et al*., 1981; Rajashekara *et al*., 2000; van den Bogaard and Stobberingh, 2000). Therefore, in consumers' minds, organic foods appear to be a safer alternative to conventional poultry.

Several studies have assessed preharvest *Salmonella* prevalence on conventional broiler farms (Renwick *et al*., 1992; Bailey *et al*., 2001; Liljebjelke *et al*., 2005; Rodriguez *et al*., 2006; Arsenault *et al*., 2007). However, very little is known about the prevalence of *Salmonella* on large-scale USDA-certified organic broiler farms. In a recent study by Siemon *et al*. (2007), the prevalence of fecal *Salmonella* in pasture chicken farms (16%) was lower than the prevalence in conventional chicken farms (30.0%). Pasture broiler farms are small operations (average of 500 birds raised per year) where chickens are

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reared outside on pasture in open-air moveable pens (Jacob *et al.*, 2008). Pasture birds are grown without subtherapeutic (growth promotion) or therapeutic antimicrobial use (i.e., considered as antibiotic-free chickens); however, they are fed commercial nonorganic diet. The USDA has placed a set of national standards the broiler production must meet to be labeled organic (Dimitri and Greene, 2002). These standards are primarily (1) birds must be raised without the use of antibiotics, (2) fed organic dietary supplements and consume all organic feed free of animal by products, and (3) have access to the outside environment (Dimitri and Greene, 2002). Organic poultry production focuses on animal health and welfare, good management practices, and product quality, whereas, conventional poultry production focuses on reducing costs and maximizing production through weight gain and feed efficiency (Sundrum, 2006).

To the best of our knowledge, this is the first study conducted to compare *Salmonella* prevalences, antimicrobial-resistant phenotypes, and dissemination in large-scale USDA-certified organic broiler chicken farms compared to conventional broiler farms.

Materials and Methods

Study design and sample collection

One poultry company in North Carolina that operates both USDA-certified organic and conventional broiler farms participated in this study. On organic broiler farms, birds were reared in medium-sized houses (~200–300 feet long) that are similar in structure to conventional farms with an all-in–all-out system, fed all USDA-certified organic feed free of antimicrobial agents and animal by-products, exposed to sunlight, have twice more area per square foot than conventional birds, and have access to the outside environment, although birds usually prefer to stay in-doors. Broiler chicks were brought to the farms at 1 day of age and were sent to slaughter at 55–60 days of age. Houses had from 3000 to 5000 birds inside, per flock. On conventional poultry farms, the broilers were reared intensively in large houses (~500–600 feet long) with an all-in–all-out system. Houses had from 15,000 to 30,000 broilers inside, and the broilers were slaughtered at 50–55 days of age. Feed-grade antibiotic (i.e., bacitracin methylene disalicylate) was routinely used at 25–50 g/ton of feed on the conventional poultry farms. Further, coccidiostats were used on the conventional farms.

A convenience sample of seven broiler farms (three organic and four conventional) were included in the study. One broiler house per farm (organic and conventional) was sampled. Each farm/house included in the study was followed for two consecutive flock cycles. Each broiler flock within a house was sampled twice; at 3–4 weeks of age and 1 week before slaughter to approximate *Salmonella* prevalence near slaughter. Appropriate dress in clean coveralls, plastic shoe covers, and lab-grade plastic gloves was used on entry to the farm. At each visit, 15 individual fresh fecal floor droppings (~5 g) were collected using a zig-zag pattern through the entire house at the organic and conventional farms. In addition, we collected feed samples ($n = 5$; 50–100 g) and drinking water ($n = 5$; 1200 mL). Feed samples consisted of feed hopper ($n = 2$) and feed lines ($n = 3$). A Feed hopper is a V-shaped container for the incoming feed to the house to provide feed to the birds through the feed lines. Feed was collected as soon as new feed is dispensed into the hopper. Water samples consisted of house main water line ($n = 1$) and in-house drinking nipples ($n = 4$). Fecal and feed samples were collected with the use of sterile plastic gloves and sealed in sterile Whirl Pak© bags (Wisconsin; Nasco, Ft. Atkinson, WI), and water samples were collected in sterile Nalgene® containers (Nalgene, Rochester, NY). All samples were put in coolers on ice and shipped to the University of Georgia Center for Food Safety for laboratory analysis within 36–48 h of collection.

Salmonella *isolation and identification*

Fecal samples were mixed thoroughly and 1 g portions were added to 9 mL tetrathionate brilliant green broth (TBG) with 2 mL iodine (Difco, Division of Becton, Dickinson and Co., Sparks, MD) and incubated for 24 h at 42°C. Two loopful of the incubated media were streaked onto two xylose-lysine-tergitol-4 agar plates (Difco) for selective differentiation via 24 h incubation at 37°C. One presumptive black colony from each positive xylose-lysine-tergitol-4 plate was tested for biochemical reaction on triple sugar iron (TSI; Difco) slant. The slants were incubated at 37°C for 24 h. Identification of suspected *Salmonella* was confirmed and serogrouped using poly-O *Salmonella*-specific antiserum (MiraVista, Indianapolis, IN). A portion of the growth from the TSI slants was transferred and streaked into nutrient agar slants (Difco) and incubated at 37°C for 24 h. Nutrient agar slants with *Salmonella* growth were stored at room temperature until shipped to The Ohio State University Infectious Disease Molecular Epidemiology Laboratory for antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE) genotypic characterization.

A 25 g portion of feed sample was mixed with 225 mL TBG with 4 mL iodine (Difco), and incubated for 24 h at 42°C. The remaining of the isolation procedure was the same as the method used for isolation from fecal samples. Water samples were cultured using two methods: (1) 100 mL aliquot of water sample was added to 200 mL TBG with 4 mL iodine, and incubated for 24 h at 42°C, and (2) using Moore swab method (Moore, 1948), where a sterile cotton swab (four-inches-by-three-feet-long gauze) was suspended in the 1000 mL of water sample with stirring and left for 24 h at room temperature (24°C), and then swab was aseptically removed, cut, and added to 225 mL TBG with 4 mL iodine, and then incubated for 24 h at 42°C. The remainder of the isolation procedure for both methods was the same as for isolation from fecal samples. *Salmonella* isolates ($n = 70$) were serogrouped using poly-O *Salmonella*-specific antiserum (MiraVista).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of *Salmonella* isolates ($n = 70$) was performed using Kirby-Bauer disk diffusion method to a panel of 16 antimicrobial agents all of which are routinely tested by the National Antimicrobial Resistance Monitoring System (CDC, 2003). The following BBL™ Sensi-Disc™ antimicrobial susceptibility test discs (Becton, Dickinson) with their respective disc potencies were used: ampicillin (Am-10 μg), amoxicillin/clavulanic acid (Ax-20/10 μg), amikacin (An-30 μg), cefoxitin (Fx-30 μg), ceftriaxone (Ce-30 μg), ceftiofur (Cf-30 μg), cephalothin (Ch-30 μg), chloramphenicol (Cl-30 μg), ciprofloxacin (Cip-5 μg), gentamicin (Gm-10 μg), kanamycin (Km-30 μg), nalidixic acid

(Nl-30 μg), streptomycin (St-10 μg), sulfamethoxazole (Su-250 μg), tetracycline (Te-30 μg), and Trimethoprim/sulfamethoxazole (Sm-1.25/23.75). Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) recommendations (NCCLS, 2002). All isolates that showed intermediate resistance were grouped with the susceptible strains to avoid overestimation of resistance. Control tests of *E. coli* ATCC 25922, *E. coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeroginosa* ATCC 27853 were regularly performed in accordance with the CLSI standards (NCCLS, 2002).

Genotypic characterization of Salmonella *using PFGE*

DNA fingerprinting of *Salmonella* isolates using PFGE were performed according to CDC PulseNet standardized protocol (Ribot *et al.*, 2006). Briefly, an overnight grown *Salmonella* cell concentration was adjusted by diluting with sterile cell suspension buffer to the OD value 1.3 to 1.4 measured at 610 nm wavelength with a spectrophotometer. Agarose-embedded cells were lysed, and intact genomic DNA was digested with 50U of *Xba*I restriction enzyme (New England Biolabs, Ipswich, MA) for 2 h at 37°C. The fragments were then separated by CHEF-DR® III system (Bio-Rad Laboratories, Hercules, CA) with the following conditions and reagents: 1% SeaKem Gold agarose (FMC BioProducts, Rockland, ME), in 0.5% Tris-borate EDTA buffer; temperature, 14°C; voltage, 6 V/cm; run time, 18 h with switch times ranging from 2.2 to 63.8 s. The PulseNet "universal" standard marker strain *Salmonella enterica* serovar Braenderup H9812 was used as a molecular reference marker and an "Out-group" strain. The gels were stained with ethidium bromide, and the DNA bands were observed under UV trans-illumination (Gel Doc™ 2000; Bio-Rad Laboratories) and gel images were captured using Quantity one 1-D analysis software (Bio-Rad Laboratories).

Fingerprint images were analyzed by Bionumerics software V. 4.61 (Applied Maths, Kortrijk, Belgium) as per the manufacturer's recommendation. Briefly, the captured gel images were normalized by aligning the lanes of the gel to a reference standard for the *Salmonella* database. To identify both the distance measure and cluster algorithm that "best" describe clustering among PFGE gel patterns, various distance (i.e., proximity) measures (e.g., Dice, Jaccard, and Matching) and clustering algorithms (e.g., complete linkage, single linkage, and Ward) were applied, and a dendrogram tree was generated. A dice coefficient index with optimization of 1.5% and position tolerance of 2.0% was used in analysis of images in this study. For clustering purposes, a threshold cut-off value of 80% was used with subclustering at 85%.

Statistical analysis

The association between fecal *Salmonella* prevalence and the study factors (farm type [organic and conventional], age group [3–4 weeks, and 1 week before slaughter], and flock cycle [first and second]) in the present study was assessed using a generalized linear model, with binomial error distribution and logit link function and adjusted for dependency within farms, using a generalized estimated equations (GEE) in STATA software version 10.1 (Stata Corp., College Station, TX). GEE is a multivariable logistic regression with a population-averaged model that adjusted for estimated correlations among the isolates in this study within a cluster (i.e., farms). The reported odds ratios (OR) from GEE was comparing the odds of *Salmonella* in a group, while adjusted for the dependence of isolates within farms, to the odds of *Salmonella* in the other group (Dohoo *et al.*, 2003).

The 16 antimicrobial-resistant *Salmonella* outcomes (binary), as well as the multidrug resistance totals (multinomial), were cross-tabulated with farm type. The proportion of *Salmonella* isolates resistant to each of the antimicrobial agents was compared by farms type using either a two-sided 2-by-2 Fisher's exact test or 2-by-*n* likelihood ratio chi-square test, as appropriate, with STATA software. Multidrug resistance (resistance $\geq$2 agents) was assessed by farm type across all isolates, using an $m \times n$ likelihood ratio chi-square test.

Results

Salmonella *prevalence*

A total of 700 samples (300 organic and 400 conventional) were collected from three organic and four conventional broiler farms within one poultry company over two consecutive flock cycles. The overall prevalence of *Salmonella* across all farms, sample types, and age group was 4.3% (13/300) in organic broiler farms compared to 28.8% (115/400) in conventional broiler farms. Table 1 shows *Salmonella* prevalence by sample type for each of the seven farms that participated in the study. The prevalence of *Salmonella* by sample type was compared between organic and conventional broiler farms as shown in Table 2. The OR for prevalence of *Salmonella* in fecal and feed samples adjusted for the dependence of isolates within farms was significantly ($p < 0.05$) higher (OR = 11.9 and 7.2, respectively) in conventional farms than in organic farms. *Salmonella* isolates from organic feed were all from the feed lines (i.e., feed pans); however, two of the isolates from conventional feed were from the feed hopper (i.e., incoming feed). The prevalence of *Salmonella* by birds' age group and by flock cycle was compared between organic and conventional broiler farms as shown in Table 3. In Table 3, the OR for

Table 1. Prevalence of *Salmonella* by Farm and Sample Type

Sample type	*Organic farms* (n = *3)*			*Conventional farms* (n = *4)*			
	CK[a]	KB[a]	PG[a]	JDK[a]	MH[a]	RS[a]	RG[a]
Feces	5 (3/60)	1.7 (1/60)	10 (6/60)	31.6 (19/60)	45 (27/60)	43.3 (26/60)	35 (21/60)
Feed	0 (0/20)	0 (0/20)	15 (3/20)	20 (4/20)	40 (8/20)	20 (4/20)	30 (6/20)
Water	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/20)

[a]CK, KB, PG, JDK, MH, RS, and RG are codes used in the study for farm identification during sample collection and analysis. Frequencies are contrasted by each farm (organic and conventional) and sample type across all age groups and flock cycles.

Table 2. Prevalence of *Salmonella* by Farm Type and Sample Type

Sample type	*Organic* (n = *300*)	*Conventional* (n = *400*)	*Odds ratio*[a]	p-*Value*[b]
Feces	5.6 (10/180)	38.8 (93/240)	11.9	<0.0001
Feed	5.0 (3/60)	27.5 (22/80)	7.2	0.007
Water	0 (0/60)	0 (0/80)	–	–

[a]Odds ratio values represent a comparison of the odds of the prevalence of *Salmonella* in fecal and feed samples, adjusted for the dependence of isolates within farms, in conventional compared to organic farms.

[b]*p*-Values are adjusted for the dependence of *Salmonella* isolates within farms by using generalized estimating equation statistic in STATA (Stata Corp.). Frequencies are contrasted by sample type across all farms, age group, and flock cycles.

prevalence of *Salmonella* in samples collected from birds 1 week before slaughter adjusted for the dependence of isolates within farms was significantly ($p < 0.05$) higher (OR = 2.2) than 3–4 weeks of age in conventional farms, whereas no significant difference ($p > 0.05$) in organic farms was found. Further, within each farm type, the OR for prevalence of *Salmonella* in samples collected from the second flock cycle, adjusted for the dependence of isolates within farms, was not significantly ($p > 0.05$) different compared to first flock cycle.

The majority of the isolates (92.9%; 65/70) were *Salmonella* serogroup C. The antisera used in the laboratory did not distinguish between C1, C2, or C3, so we only know that it was group C. The remaining five isolates were serogroup B.

Antimicrobial resistance

Antimicrobial susceptibility testing was performed on 70 selected *Salmonella* isolates (12 from organic and 58 from conventional farms) out of 128 isolates that represented the majority from both types of farms. These isolates were selected to represent all the farms, farm type, and sample type (if available). The individual antimicrobial resistance *Salmonella* phenotypes cross-tabulated by farm type are shown in Table 4.

In isolates from organic farms, 25% (3/12) were pansusceptible, 33.3% (4/12) had single-agent-resistance, and 41% (5/12) were resistant to two or more antimicrobial agents. In conventional farms, 1.7% (1/58) of the isolates were pansusceptible, 36.2% (21/58) were single-agent-resistant, and 62% (36/58) were resistant to two or more antimicrobial agents. The distribution of multidrug-resistant *Salmonella* phenotypes by farm type is shown in Table 5. The proportion of multidrug-resistant *Salmonella* isolates differed significantly by farm type ($p = 0.001$). The predominant resistance patterns among *Salmonella* isolates from conventional farms were ampicillin- streptomycin-amoxicillin/clavulanic acid-cephalothin-ceftiofur-cefoxitin (AmStAxChCfFx; 39.7%: 25/58), and streptomycin (36.2%; 21/58), whereas the predominant *Salmonella* resistance patterns from organic farms were pansusceptible (25%; 3/12), and streptomycin (25%; 3/12).

Pulsed-field gel electrophoresis

To assess genotypic relatedness, PFGE was used to analyze 70 *Salmonella* isolates. A total of four main PFGE clusters were found in this study (Fig. 1a, b). Cluster type 1 (with two subclusters 1A and 1B) was the most predominant. This cluster was composed of isolates that originated from both production types and various resistance phenotypes. The PFGE patterns showed the highly clonal nature of the *Salmonella* isolates within and between the organic and conventional broiler farms. In addition, clonality of isolates between feed and fecal samples was detected at individual farm levels

Table 3. Prevalence of *Salmonella* by Farm Type and Both Age Group and Flock Cycle

	Organic (n = *300*)	*Conventional* (n = *400*)
Age group		
3–4 weeks	4.7 (7/150)	21.0 (42/200)
1 week before slaughter	4.0 (6/150)	36.5 (73/200)
Odds ratio[a]	0.85	2.2
p-Value[b]	0.766	0.001
Flock cycle		
Flock cycle #1	3.3 (5/150)	30.5 (61/200)
Flock cycle #2	5.3 (8/150)	27.0 (54/200)
Odds ratio[c]	1.6	0.84
p-Value[b]	0.4	0.433

[a]Odds ratio values represent a comparison of the odds of the prevalence for each age group within organic and conventional *Salmonella* isolates.

[b]*p*-Values are adjusted for the dependence of *Salmonella* isolates within farms by using generalized estimating equation statistic in STATA (Stata corp.). Frequencies are contrasted by age group across all farms, sample type, and flock cycles.

[c]Odds ratio values represent a comparison of the odds of the prevalence of *Salmonella*, within each farm type, in samples collected from the second flock cycle adjusted for the dependence of isolates within farms in conventional compared to organic farms.

Table 4. Phenotypic Resistance of *Salmonella* Isolates Between Farm Type

	No. (%) of Salmonella *isolates across farm type*		
Antimicrobial	*Organic* (n = *12*)	*Conventional* (n = *58*)	p-*Value*[a]
Amikacin	0 (0)	0 (0)	–
Amoxicillin/ clavulanic acid	5 (41.7)	32 (55.2)	0.528
Ampicillin	6 (50.0)	33 (56.9)	0.754
Cephalothin	5 (41.7)	33 (56.9)	0.361
Cefoxitin	1 (8.3)	32 (55.2)	0.004
Ceftiofur	1 (8.3)	31 (53.5)	0.004
Ceftriaxone	0 (0)	5 (8.6)	0.579
Chloramphenicol	0 (0)	0 (0)	–
Ciprofloxacin	0 (0)	0 (0)	–
Gentamicin	2 (16.7)	1 (1.7)	0.074
Kanamycin	1 (8.3)	1 (1.7)	0.316
Nalidixic acid	0 (0)	0 (0)	–
Streptomycin	7 (58.3)	53 (91.4)	0.010
Sulfisoxazole	1 (1.72)	3 (25.0)	0.014
Tetracycline	4 (33.3)	4 (6.9)	0.025
Trimethoprim/ sulfamethoxazole	0 (0)	0 (0)	–

[a]*p*-Values are based on Fisher's exact test of the differences in risk between farm type.

Table 5. Number and Percentage of *Salmonella* Isolates with Antimicrobial Resistance Pattern by Farm Type

Antimicrobial resistance pattern	*No. (%) of organic isolates (n = 12)*	*No. (%) of conventional isolates (n = 58)*	*Total no. (%) of isolates (n = 70)*
AmStSuTeAxChKmCfFx	1 (8.3)	1 (1.7)	2 (2.9)
AmStAxChCeCfFx	0 (0)	3 (5.2)	3 (4.3)
AmStSuTeAxChGm	1 (8.3)	0 (0)	1 (1.4)
AmStTeAxChCfFx	0 (0)	1 (1.7)	1 (1.4)
AmAxChCeCfFx	0 (0)	2 (3.5)	2 (2.9)
AmStAxChCfFx	0 (0)	23 (39.7)	23 (32.9)
AmStSuTeChGm	1 (8.3)	0 (0)	1 (1.4)
AmAxChCfFx	0 (0)	1 (1.7)	1 (1.4)
AmStAxChFx	0 (0)	1 (1.7)	1 (1.4)
AmStTeAxCh	1 (8.3)	0 (0)	1 (1.4)
AmAxCh	1 (8.3)	0 (0)	1 (1.4)
AmCh	0 (0)	1 (1.7)	1 (1.4)
StKm	0 (0)	1 (1.7)	1 (1.4)
StTe	0 (0)	2 (3.5)	2 (2.9)
St	3 (25.0)	21 (36.2)	24 (34.3)
Am	1 (8.3)	0 (0)	1 (1.4)
Pansusceptible	3 (25.0)	1 (1.7)	4 (5.7)

Am, ampicillin; Ax, amoxicillin/clavulanic acid; An, amikacin; Fx, cefoxitin; Ce, ceftriaxone; Cf, ceftiofur; Ch, cephalothin; Cl, chloramphenicol; Cip, ciprofloxacin; Gm, gentamicin; Km, kanamycin; NI, nalidixic acid; St, streptomycin; Su, sulfasoxazole; Te, tetracycline, Sm, Trimethoprim/sulfamethoxazole.

implying potential transmission of *Salmonella* via contaminated feed.

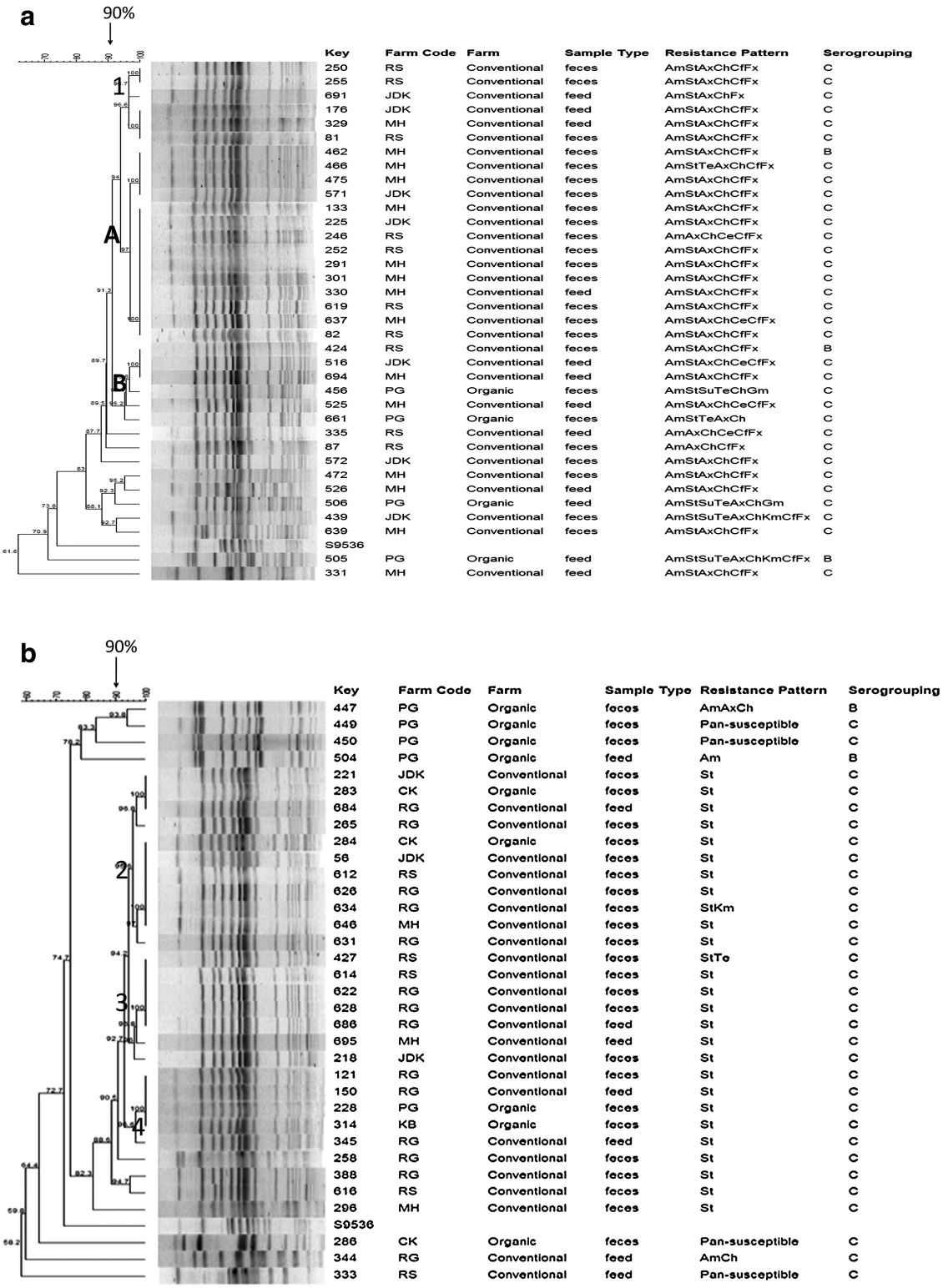
Discussion

To the best of our knowledge, this present study is the first to investigate the prevalence of *Salmonella*, determine antimicrobial-resistant phenotypes of *Salmonella*, and investigate *Salmonella* dissemination in large-scale USDA-certified organic broiler farms compared to conventional broiler farms owned by the same company.

In the current study, the overall prevalence of *Salmonella* and fecal shedding of *Salmonella* was lower in organic farms than in conventional farms. Further, the overall prevalence of *Salmonella* by age group (3–4 weeks and 1 week before slaughter) was significantly different within conventional farms, but not significant in organics. There was no significance difference in the prevalence of *Salmonella* by flock cycle for either farm type. In feed, the prevalence of contamination with *Salmonella* was lower in organic farms than in conventional farms. Contaminated feed at the organic farms was only present in the feed lines (i.e., feed pans), which is likely due to feed contamination with the birds' fecal droppings. *Salmonella* was present in feed samples from the feed hopper (i.e., incoming feed) as well as in the feed line samples at the conventional farms. This may indicate that incoming conventional feed was contaminated with *Salmonella*. Some *Salmonella* cells could be injured during feed production (e.g., heating step during feed pelleting) of conventional feed. Organic feed was produced without pelleting (i.e., mash). We used a selective enrichment broth (TBG) to recover *Salmonella* from both types of feed samples. This method might underestimate the true prevalence of cultivable *Salmonella*, mostly in conventional feed samples. Despite the use of selective broth, we recovered higher proportions of *Salmonella* from conventional feed than from organic feed samples.

The presence of *Salmonella* in broiler feed and feed ingredients (e.g., bone, meat, and fish meal) is well documented (Allred *et al.*, 1967; Stott *et al.*, 1975; Furuta *et al.*, 1980; Bailey *et al.*, 2001; Maciorowski *et al.*, 2004). Bailey *et al.* (2001) reported *Salmonella* prevalence of 2.33% (6/258) and 2.28% (6/263) in feed hopper and feeder (i.e., feedlines) in conventional broiler farms. In our study, it was common to find fecal droppings in the feed pans at both types of farms; however, due to the higher prevalence of fecal shedding of *Salmonella* at the conventional farms (as our results indicated), conventional feed was more likely to be contaminated with fecal *Salmonella* and horizontally spread this pathogen to other birds within the same house. In a previous study by Siemon *et al.* (2007), the authors reported that fecal *Salmonella* prevalence in conventional bird flocks (30%; 125/419) was significantly higher than in pasture flocks (16%; 83/512). The conventional and pasture farms were owned by different companies. Pasture poultry farms are small operations where birds are reared on pasture and usually not classified as USDA-certified organic birds (Jacob *et al.*, 2008). Various overall estimates of *Salmonella* prevalence in conventional broiler farms in the United States were reported in several studies ranged from 10% to 26% (Bailey *et al.*, 2001; Liljebjelke *et al.*, 2005; Rodriguez *et al.*, 2006). These prevalence estimates usually vary between studies due to seasonal effects, differences in the hatchery sources, feed composition, vaccination programs, and flock-disease status. It has been shown that *Salmonella* is found in the hatcheries due to vertical and/or horizontal transmission of this pathogen (Cox *et al.*, 1990; Bailey *et al.*, 1992; Bailey *et al.*, 1994; Cox *et al.*, 1995). Broiler chicks (organic or conventional) in this study were originated from the same conventional hatchery, but most likely from different breeder flocks. The cooperator poultry company has no information on which broiler birds came from which breeder flocks (personal communication with the poultry company's consultant veterinarian). There was no sample collection conducted at the arrival of chicks to the farms, as the main interest of the study was to determine *Salmonella* prevalence, *Salmonella* antimicrobial resistance profiles, and dissemination in the middle of the production cycle as well as during the last week before slaughter to assess the preharvest food safety risk associated with organic broilers compared with conventional broilers. Organic birds were raised under organic conditions starting at day 1 when they arrived at the farms. If we assume that the distribution of chicks at both farm types was random, then each farm (organic or conventional) would have received broiler birds representing the multiple breeder flocks that supplied the hatchery.

The prevalence of *Campylobacter* in large-scale USDA-certified organic and conventional farms in Ohio was estimated at 89% and 66%, respectively (Luangtongkum *et al.*, 2006). The authors collected their study samples from intestinal tracts of organic and conventional birds at processing plants. In a different species, prevalence of *Salmonella* was higher in antibiotic-free (niche-market and out-door) swine farms than in conventional (intensive and indoor) swine farms (Gebreyes *et al.*, 2006; Gebreyes *et al.*, 2008).



Key	Farm Code	Farm	Sample Type	Resistance Pattern	Serogrouping
250	RS	Conventional	feces	AmStAxChCfFx	C
255	RS	Conventional	feces	AmStAxChCfFx	C
691	JDK	Conventional	feed	AmStAxChFx	C
176	JDK	Conventional	feed	AmStAxChCfFx	C
329	MH	Conventional	feed	AmStAxChCfFx	C
81	RS	Conventional	feces	AmStAxChCfFx	C
462	MH	Conventional	feces	AmStAxChCfFx	B
466	MH	Conventional	feces	AmStTeAxChCfFx	C
475	MH	Conventional	feces	AmStAxChCfFx	C
571	JDK	Conventional	feces	AmStAxChCfFx	C
133	MH	Conventional	feces	AmStAxChCfFx	C
225	JDK	Conventional	feces	AmStAxChCfFx	C
246	RS	Conventional	feces	AmAxChCeCfFx	C
252	RS	Conventional	feces	AmStAxChCfFx	C
291	MH	Conventional	feces	AmStAxChCfFx	C
301	MH	Conventional	feces	AmStAxChCfFx	C
330	MH	Conventional	feed	AmStAxChCfFx	C
619	RS	Conventional	feces	AmStAxChCfFx	C
637	MH	Conventional	feces	AmStAxChCeCfFx	C
82	RS	Conventional	feces	AmStAxChCfFx	C
424	RS	Conventional	feces	AmStAxChCfFx	B
516	JDK	Conventional	feed	AmStAxChCeCfFx	C
694	MH	Conventional	feed	AmStAxChCfFx	C
456	PG	Organic	feces	AmStSuTeChGm	C
525	MH	Conventional	feed	AmStAxChCeCfFx	C
661	PG	Organic	feces	AmStTeAxCh	C
335	RS	Conventional	feed	AmAxChCeCfFx	C
87	RS	Conventional	feces	AmAxChCfFx	C
572	JDK	Conventional	feces	AmStAxChCfFx	C
472	MH	Conventional	feces	AmStAxChCfFx	C
526	MH	Conventional	feed	AmStAxChCfFx	C
506	PG	Organic	feed	AmStSuTeAxChGm	C
439	JDK	Conventional	feces	AmStSuTeAxChKmCfFx	C
639	MH	Conventional	feces	AmStAxChCfFx	C
S9536					
505	PG	Organic	feed	AmStSuTeAxChKmCfFx	B
331	MH	Conventional	feed	AmStAxChCfFx	C

Key	Farm Code	Farm	Sample Type	Resistance Pattern	Serogrouping
447	PG	Organic	feces	AmAxCh	B
449	PG	Organic	feces	Pan-susceptible	C
450	PG	Organic	feces	Pan-susceptible	C
504	PG	Organic	feed	Am	B
221	JDK	Conventional	feces	St	C
283	CK	Organic	feces	St	C
684	RG	Conventional	feed	St	C
265	RG	Conventional	feces	St	C
284	CK	Organic	feces	St	C
56	JDK	Conventional	feces	St	C
612	RS	Conventional	feces	St	C
626	RG	Conventional	feces	St	C
634	RG	Conventional	feces	StKm	C
646	MH	Conventional	feces	St	C
631	RG	Conventional	feces	St	C
427	RS	Conventional	feces	StTe	C
614	RS	Conventional	feces	St	C
622	RG	Conventional	feces	St	C
628	RG	Conventional	feces	St	C
686	RG	Conventional	feed	St	C
695	MH	Conventional	feed	St	C
218	JDK	Conventional	feces	St	C
121	RG	Conventional	feces	St	C
150	RG	Conventional	feed	St	C
228	PG	Organic	feces	St	C
314	KB	Organic	feces	St	C
345	RG	Conventional	feed	St	C
258	RG	Conventional	feces	St	C
388	RG	Conventional	feces	St	C
616	RS	Conventional	feces	St	C
296	MH	Conventional	feces	St	C
S9536					
286	CK	Organic	feces	Pan-susceptible	C
344	RG	Conventional	feed	AmCh	C
333	RS	Conventional	feed	Pan-susceptible	C

FIG. 1. Dendogram of pulsed-field gel electrophoresis patterns of *Salmonella* isolates ($n = 70$) recovered from feces and feed sampled from organic and conventional broiler farms. (**a**) Dendogram with highly antimicrobial-resistant isolates, and (**b**) dendogram with less resistant or pansusceptible isolates. The tree of relative genetic similarity was constructed based on the Dice method; scale at 100 means identical. Four main clusters and two subclusters were detected: 1 (A and B), 2, 3, and 4. Keys S9638 and S9563 were "out-group" *Salmonella* isolates from a different study, but from the same serogroups as our study. The "out-group" isolates used to generate clades of isolates within our study.

We did not detect *Salmonella* in our water samples using either isolation method. In Lilebjelke *et al.* (2005), the authors found one *Salmonella* isolate out of 56 water samples collected from conventional broiler houses. They used a similar enrichment/isolation protocol (100 mL of water added to 200 mL of TBG with 4 mL iodine) as in our study. In another study, Bailey *et al.* (2001) reported a *Salmonella* prevalence of 1.4% (10/731) in water sampled from house waterlines. We believe that if *Salmonella* was present in the water samples, it would have been at very low levels below the detection limit (10 CFU/mL) of the two isolation/culture methods used in the present study.

A comparison between *Salmonella* prevalence in organic and conventional broiler meat at retail stores was examined in several studies. Lestari *et al.* (2009) reported *Salmonella* prevalence in organic (20.8%) and conventional (22%) chicken carcass samples collected from 27 retail stores in Baton Rouge, Louisiana. The authors found that the difference was not statistically significant between both types of samples. It was found in other studies that *Salmonella* prevalence in broiler chicken meat from conventional birds was lower than that in chicken meat from organic birds (Bailey and Cosby, 2005; Cui *et al.*, 2005). Cui *et al.* (2005) reported *Salmonella* prevalence in organic and conventional chicken meat, collected from retail stores in Maryland, at 61% and 44%, respectively. It was not clear in the two studies (Cui *et al.*, 2005; Lestari *et al.*, 2009) whether organic chicken carcasses were from USDA-certified organic birds, pasture birds, or free-range birds. In Bailey and Cosby (2005), the authors reported an overall *Salmonella* prevalence of 31% and 25% in free-range and all-natural chicken meat, respectively, obtained from retail stores. Further, it was reported that the prevalence of *Salmonella* in their USDA-certified free-range chicken samples was 60%. In that study, the prevalence of *Salmonella* estimates at 31% and 25% were compared to the Food Safety and Inspection Service *Salmonella* reports during chicken processing (USDA, 2004). The *Salmonella* prevalence was 12.8% in conventional chicken carcasses according to the Food Safety and Inspection Service estimates in 2004, and most recently the postchill prevalence of *Salmonella* was reported at 5.2% ($n = 2114$) (USDA, 2009).

The overall prevalence of individual and multidrug antimicrobial resistance was higher in *Salmonella* isolates from conventional broiler farms than in those from organic broiler farms. Multidrug resistance (resistance to two or more antibiotics) was more frequent in *Salmonella* isolates from conventional broiler farms (55.2%) compared with organic farms (41.6%). Forty-three percent (25/58) of isolates from conventional farms were resistance to six drugs (AmStAxChCfFx). The prevalence of multidrug-resistant *Salmonella* at conventional and organic farms in the study of Siemon *et al.* (2007) was only 35% and 30.2%, respectively. Conventional farms in the present study used bacitracin methylene disalicylate antibiotic supplement in feed at a subtherapeutic level to prevent necrotic enteritis infections caused by *Clostridium perfringens* and as a growth promoter. In contrast, organic farms did not use any antibiotics as a supplement or as a treatment. Necrotic enteritis is a multifactorial disease with a complex etiology. Although it would be interesting to compare the prevalence of *Clostridium perfringens* and clinical evidence of necrotic enteritis in both farm types, we did not assess that as it was beyond the scope of our study. The predominant resistance patterns in *Salmonella* isolates from conventional farms were AmStAxChCfFx and single-resistance to streptomycin. The predominant multidrug resistance pattern in Siemon *et al.* (2007) in conventional farms was AmStAxCSuTe (C, chloramphenicol; Su, sulfasoxazole; Te, tetracycline), which was similar to ours except for the cephalosporins class resistance. Many of the multidrug-resistant *Salmonella* isolates in our study have unique cephalosporins drug-class resistance pattern (cephalothin, cefoxitin, ceftiofur, and ceftriaxone). From a public health standpoint, ceftriaxone is considered to be a drug of choice to treat human cases of salmonellosis, especially in children. Ceftriaxone resistance in *Salmonella* in chicken is not common as it was found in 1% isolates out of 1121 (Gray *et al.*, 2004). In our study, resistance to ceftriaxone was found in 8.6% (5/58) of *Salmonella* isolates, all were from conventional farms. Cui *et al.* (2005) found that 3.3% and 54% of *Salmonella* isolates from organic and conventional retail chicken were resistant to cephalothin-cefoxitin-ceftiofur, respectively. Similar findings on decreased susceptibility to cephalosporins in *Salmonella* isolates from retail organic and conventional chickens were reported by Lestari *et al.* (2009). Single resistance to streptomycin was higher (91.4%; 53/58) among isolates from conventional farms in the current study than 0.5% in Siemon *et al.* (2007) conventional farm samples. In our organic farms, the predominant *Salmonella* resistance patterns were pansusceptible (25%; 3/12) and streptomycin (25%; 3/12). Siemon *et al.* (2007) reported higher percentage of pansusceptible *Salmonella* isolates from pasture broilers (57%) and lower for streptomycin (3.1%) compared to our findings in organic broilers. The prevalence of individual and multidrug-resistant *Salmonella* from our organic farms need to be interpreted with caution as the number of the isolates ($n = 12$) from the collected samples ($n = 300$) was low. However, this suggested that the preharvest risk of *Salmonella* shedding in organic broiler farms was much lower than in the conventional broiler farms. According to the recently released NARMS (2006) report, *Salmonella* prevalence in retail chicken breast was 12.7% with 2.0% resistant to ACSSuTAuCf (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, tetracycline, amoxicillin-clavulanic acid, and ceftiofur).

The PFGE genotyping findings suggested a highly clonal pattern for *Salmonella* isolated from organic and conventional broiler farms. *Salmonella* dissemination was not only within individual farms, but also between farms despite the differences in antimicrobial resistance patterns between the isolates. The resistance phenotypes in some or most cases may be inducible via antimicrobial use. Designated poultry company personnel were allowed to work with multiple farms (both organic and conventional) to supervise broiler production and assist growers. Further, farms sampled were located within a 30-mile radius. The high clonality between isolates of fecal and feed origin is significant as it may imply one additional route of dissemination of *Salmonella* strains to multiple farms via contaminated feed. Another possible explanation for the high clonality among the isolates is the lack of diversity among *Salmonellae* in our study. Around 93% of our isolates were *Salmonella* group C. Further, according to the literature, the majority (80%–90%) of *Salmonella* isolated from broiler birds are *Salmonella* Kentucky, which belongs to group C. However, due to the budget limitations for this study, we did not serotype any of the isolates. In another study, Lestari

et al. (2009) found a very similar PFGE pattern in *Salmonella* isolates from organic and conventional chicken meat collected at retail stores.

Conclusions

Our study aimed to compare the prevalence of *Salmonella* in large-scale USDA-certified organic broiler farms relative to conventional broiler farms, within the same poultry company in North Carolina. The results of our study suggest that within this poultry company, prevalence of fecal *Salmonella* was lower in certified-organic birds than in conventionally raised birds. In addition, the prevalence of antimicrobial-resistant *Salmonella* was higher in conventionally raised birds than in certified-organic birds. Multidrug-resistant *Salmonella* strains were isolated from organic broiler farms even with the absence of antimicrobial selection pressure. Similarities in PFGE patterns for *Salmonella* isolates from both farm types may suggest that these organisms were circulating within the poultry company's farms or due to the lack of diversity among the *Salmonella* isolates in our study. Further longitudinal studies are needed to determine *Salmonella* loads quantitatively and verify *Salmonella* serotypes in multiple large-scale certified-organic on-farm operations and at processing plants.

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Disclosure Statement

No competing financial interests exist.

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