Lower prevalence of antibiotic-resistant *Salmonella* on large-scale U.S. conventional poultry farms that transitioned to organic practices

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H I G H L I G H T S

- Studied levels of antibiotic-resistant *Salmonella* on newly organic poultry farms
- Tested litter, water and feed from 10 newly organic and 10 conventional houses
- Multidrug resistance was significantly lower in newly organic poultry houses.
- Ceasing antibiotic use in US poultry can lead to lower levels of on-farm resistance.

A R T I C L E   I N F O

A B S T R A C T

As a result of the widespread use of antibiotics in large-scale U.S. poultry production, a significant proportion of *Salmonella* strains recovered from conventional poultry farms and retail poultry products express antibiotic resistance. We evaluated whether large-scale poultry farms that transitioned from conventional to organic practices and discontinued antibiotic use were characterized by differences in the prevalence of antibiotic-resistant *Salmonella* compared to farms that maintained conventional practices. We collected poultry litter, water and feed samples from 10 newly organic and 10 conventional poultry houses. Samples were analyzed for *Salmonella* using standard enrichment methods. Isolates were confirmed using standard biochemical tests and the Vitek®2 Compact System. Antimicrobial susceptibility testing was performed by Sensititre® microbroth dilution. Data were analyzed using Fisher's exact test and generalized linear mixed models. We detected *Salmonella* in both conventional and newly organic poultry houses. *Salmonella* Kentucky was the predominant serovar identified, followed by *S.* Orion, *S.* Enteritidis, *S.* Gostrup and *S.* Infantis. Among *S.* Kentucky isolates (n = 41), percent resistance was statistically significantly lower among isolates recovered from newly organic versus conventional poultry houses for: amoxicillin–clavulanate ($p = 0.049$), ampicillin ($p = 0.042$), cefoxitin ($p = 0.042$), ceftiofur ($p = 0.043$) and ceftriaxone ($p = 0.042$). Percent multidrug resistance (resistance to ≥3 antimicrobial classes) was also statistically significantly lower among *S.* Kentucky isolates recovered from newly organic poultry houses (6%) compared to those recovered from conventional houses (44%) ($p = 0.015$). To our knowledge, these are the first U.S. data to show immediate, on-farm changes in the prevalence of antibiotic-resistant *Salmonella* when antibiotics are voluntarily withdrawn from large-scale poultry facilities in the United States.

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1. Introduction

Salmonella is a leading bacterial cause of foodborne illness, responsible for an estimated 1.2 million cases of gastroenteritis annually in the United States (Scallan et al., 2011) and an estimated 93.8 million cases globally each year (Majowicz et al., 2010). The consumption of contaminated poultry products remains an important risk factor for salmonellosis (Guo et al., 2011). From 2002–2011, the

percentage of tested retail chicken products that were *Salmonella*-positive in the United States ranged from 10% to 20% (U.S. Food and Drug Administration, 2013). In a recent study by Guo et al. (2011), the authors estimated that 48% of reported domestically-acquired salmonellosis cases in the United States (between 1998 and 2003) were attributed to chicken.

As a result of the use of antibiotics for therapeutic, prophylactic and non-therapeutic purposes in conventional poultry production, antibiotic-resistant and multi-drug resistant strains of *Salmonella* are also prevalent on retail chicken products (U.S. Food and Drug Administration, 2013). In 2011, 21% of all *Salmonella* serotypes recovered from tested retail chicken products in the United States were multi-drug resistant to six or seven antimicrobial classes (U.S. Food and Drug Administration, 2013). Meanwhile, reports of highly drug-resistant *Salmonella* infections among humans are emerging in different regions of the world (Dutil et al., 2010; Le et al., 2011, 2013; Mulvey et al., 2013). While the sources of these infections are not always easily discernible, these data point towards a worrisome progression towards fewer therapeutic options for treating cases of salmonellosis (Collignon, 2013).

These data are increasingly highlighted in mainstream media and have influenced consumer demand for organic poultry (Oberholtzer et al., 2006) which is perceived to be safer than conventional poultry (Crandall et al., 2009). This consumer demand has bolstered organic poultry production, making poultry one of the fastest growing sectors of U.S. organic products (Fanatico et al., 2009; Oberholtzer et al., 2006). Retail sales of organic poultry quadrupled between 2003 and 2006 and reached nearly $200 million in 2008 (Oberholtzer et al., 2006).

Recent cross-sectional studies have evaluated whether retail organic poultry products and organic poultry farms are characterized by differing levels of susceptible and antibiotic-resistant *Salmonella* compared to their conventional counterparts (Alali et al., 2010; Cui et al., 2005; Lestari et al., 2009). Alali et al. (2010), for instance, showed that the prevalence of susceptible and antibiotic-resistant *Salmonella* was lower in certified-organic broilers compared to conventional broilers produced by the same company in North Carolina. Cui et al. (2005) found a higher prevalence of susceptible *Salmonella* on retail organic chickens compared to conventional chickens, and a lower prevalence of antibiotic-resistant *Salmonella* Typhimurium on organic retail chickens compared to their conventional counterparts.

However, to our knowledge, no studies have evaluated changes in the prevalence of antibiotic-resistant *Salmonella* on large-scale conventional poultry farms that adopted organic practices, discontinued using antibiotics, and continued as large-scale farms that produced certified organic broilers. The objective of this study was to evaluate whether farms that transitioned from large-scale conventional broiler production to large-scale organic broiler production were different with regard to the prevalence of antibiotic-resistant *Salmonella* compared to farms that maintained conventional production practices.

2. Materials and methods

2.1. Study sites

Two types of poultry farms were included in this study: large-scale conventional broiler farms that were maintaining standard industry practices and using antibiotics (n = 5), and large-scale (previously conventional) broiler farms that had just received organic certification (by a state agency accredited by the U.S. National Organic Program) and were producing their first flock of certified organic broilers (n = 5). All participating farms were located in the Mid-Atlantic United States and were operating under the guidance of one feed mill that produced both conventional and certified organic poultry feeds, each in separate buildings of the mill. Two poultry houses from each farm were included in the study for a total of 20 poultry houses.

Poultry houses on the conventional farms were an average of 15.7 years old, 407 feet long, and 44.5 feet wide (Sapkota et al., 2011). The average number of broiler chicks in the tested flocks (when the chicks arrived) was 30,800 and the average cumulative mortality rate of the tested flocks was 2.51%. An all-in-all-out system was employed in each conventional poultry house and the average age of the flock was 36 days when testing occurred. The following antimicrobials were used in the feed at each of the conventional poultry houses: bacitracin (50 g/t), virginiamycin (15 g/t), roxarsone (45.35 g/t), salinomycin (60 g/t), nicarbazin (0.0125%) and decoquinate (27.2 g/t). Gentamicin was used at the hatcheries that supplied chicks to conventional poultry houses, and bacitracin, virginiamycin, roxarsone and salinomycin were used at the breeder farms that supplied the eggs to the hatcheries.

Poultry houses on the newly organic farms were similar in structure to the conventional farms. They were an average of 8.8 years old, 500 feet long and 46.8 feet wide (Sapkota et al., 2011). The average number of broiler chicks in the tested flocks (when the chicks arrived) was 22,608 (25% less than that of the conventional poultry houses) and the average cumulative mortality rate of the tested flocks was 4.72%. An all-in-all out system was employed in each newly organic poultry house and the average age of the flock was 36 days when testing occurred. All feed components used in the newly organic poultry houses contained no antibiotics, other animal drugs, slaughter byproducts or genetically modified organisms. An outdoor access area was provided adjacent to newly organic poultry houses; however, the farm managers relayed to us that the birds rarely chose to venture outside. Additional details regarding the U.S. National Organic Program standards for broiler production appear in the Appendix of Sapkota et al. (2011).

2.2. Sample collection

A total of 60 poultry litter samples, 40 water samples and 20 feed samples were aseptically collected – in sterile Whirl-Pak® collection bags (Nasco, Fort Atkinson, WI) – from the 20 participating poultry houses from March to June 2008. In each poultry house, litter samples (500 g) were collected from three randomly selected areas from the top 1 to 5 cm of litter; water samples (500 mL) were collected from 1) raw source water before filtration or UV treatment and 2) finished water present in the waterlines; and one feed sample (300 g) was retrieved from the central feed hopper. All samples were shipped overnight at 4 °C and processed within 24 h.

2.3. Isolation

10 g of each poultry litter and feed sample was pre-enriched in a 1:10 weight to volume dilution of 100 mL of Lactose Broth (Becton Dickinson & Co., Franklin Lakes, NJ) for 24 h at 37 °C. A 1 mL aliquot of the Lactose Broth suspension was then transferred to 15 mL of Hajna Tetrathionate Broth (Becton Dickinson & Co., Franklin Lakes, NJ) supplemented with a prepared iodine solution and incubated overnight at 37 °C. A 10 μL loopful of the enrichment culture was then streaked onto XLT4 Agar (Becton Dickinson & Co., Franklin Lakes, NJ) and incubated overnight at 37 °C. Presumptive colonies of *Salmonella* spp. appeared on XLT4 as black colonies with yellow halos. *Salmonella*-negative samples underwent a secondary enrichment: the samples were incubated at room temperature on the bench top for an additional 5 days before being sub-cultured onto XLT4. Positive colonies were streaked onto Brain Heart Infusion (BHI) (Becton Dickinson & Co., Franklin Lakes, NJ) agar for purification and incubated overnight at 37 °C. A generous colony swab was collected from each BHI purification plate and archived at −80 °C in *Brucella* Broth (Becton Dickinson & Co., Franklin Lakes, NJ) with 15% glycerol.

Isolation of *Salmonella* spp. from water samples was carried out by membrane filtration. One hundred milliliters of water was filtered through a 0.45 μm, 47 mm mixed cellulose ester filter (Millipore,