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ORIGINAL ARTICLE

Salmonella enterica isolates from pasture-raised poultry exhibit antimicrobial resistance and class I integrons

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Abstract

Aims: While considerable foodborne pathogen research has been conducted on conventionally produced broilers and turkeys, few studies have focused on free-range (organic) or pastured poultry. The current surveillance study was designed to isolate, identify and genetically characterize *Salmonella* from pastured poultry farm environment and from retail samples.
Methods and Results: In this study, 59 isolates were collected from two pastured poultry farms (n = 164; pens, feed, water and insect traps) and retail carcasses (n = 36) from a local natural foods store and a local processing plant. All isolates were serotyped and analysed phenotypically (antimicrobial resistance profiles) and genotypically (DNA fingerprints, plasmid profiles and integron analysis). *Salmonella* enterica was detected using standard microbiological methods. *Salmonella* Kentucky was the most prevalent serotype detected from the sampled sources (53%), followed by *Salmonella* Enteritidis (24%), Bareilly (10%), Mbandaka (7%), Montevideo (5%) or Newport (2%). All isolates were resistant to sulfisoxazole and novobiocin, and the majority (40/59) possessed class I integrons shown by PCR detection. Each *Salmonella* serotype elicited a distinct pulsed-field gel electrophoresis fingerprint profile, and unique differences were observed among the serotypes.
Conclusions: The findings of this study show that *Salmonella* serotypes isolated from pasture-raised poultry exhibit antimicrobial resistance and class I integrons.
Significance and Impact of the Study: This study demonstrates that despite the cessation of antibiotic usage in poultry production, antibiotic resistant *Salmonella* may still be recovered from the environment and poultry products.

Introduction

Salmonella is one of the main causes of foodborne illness in the United States and worldwide (Wegener *et al.* 2003; Zhao *et al.* 2008). Nearly 800 000–4 million *Salmonella* infections occur annually in the United States alone (Jain and Chen 2006). Poultry and poultry products are the most common sources of infection, accounting for 29% of all *Salmonella* infections (Braden 2006; Jain and Chen 2006; Linam and Gerber 2007). Poultry products marketed as free-range, natural and organic have greatly increased in popularity and can be sold for increased profit (Fanatico *et al.* 2007; Jacob *et al.* 2008). The majority of consumers believe that organic and natural products are healthier and safer than conventionally produced products (Harper and Makatouni 2002; Magnusson *et al.* 2003). However, little is known about the prevalence and characteristics of foodborne pathogens in nonconventional poultry products.

While certified organic products are supposed to meet all USDA organic certification standards, regular organic, free-range and pastured poultry rearing systems may

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represent various rearing conditions (Bailey and Cosby 2005). These poultry systems allow flocks access to the outdoors and often limit or avoid the use of antimicrobials, which may pose an increased food safety risk (Bailey and Cosby 2005; Cui *et al.* 2005; Miranda *et al.* 2008). Unlike conventional poultry, where birds are reared in large barns, pastured poultry are reared outside on pasture in smaller, open-air, movable pens (Siemon *et al.* 2007). An increase in microbiological transmission routes exists on nonconventional poultry farms, including handlers, flies, rodents, wildlife, and other farm animals and birds (Rodenburg *et al.* 2004; Meerburg *et al.* 2005).

Factors thought to play major roles in the spread of antimicrobial resistance include the misuse of antimicrobials as well as bacterial genetic factors including mobile genetic elements, such as plasmids and transposons (Barlow *et al.* 2004; Fluit and Schmitz 2004; Krauland *et al.* 2009). Integrons, although nonmobile themselves, are often present on transposons and plasmids and have been linked to the spread of antimicrobial resistance because of their ability to excise and integrate resistance-gene cassettes from the environment or other bacteria (Barlow *et al.* 2004; Nogrady *et al.* 2006; Walsh *et al.* 2007; Martin *et al.* 2008; Krauland *et al.* 2009). Integrons have been identified among clinical isolates, in farm animals and in aquatic environments (Rosser and Young 1999; Liebana *et al.* 2002; Martin *et al.* 2008).

While selective pressure on micro-organisms from antimicrobials can lead to an increase in resistant organisms, it is unknown whether resistant organisms are actually linked to antimicrobial usage in any particular animal production system (Singer and Hofacre 2006). Because many micro-organisms naturally produce antimicrobials in the environment, there may be a background population of resistant bacteria and exchange of drug resistance genes between organisms, which may be a contributing factor to the emergence of resistant pathogens on poultry farms (Singer *et al.* 2007). Consequently, prevalence and resistance information on *Salmonella* from poultry reared without antimicrobials may provide a baseline for resistance in farm poultry populations. Because of limited information available on pasture-raised poultry (Bailey and Cosby 2005), the present study was designed to assess the prevalence and spread of drug resistant isolates of *Salmonella* in this system on the farm and after processing.

Materials and methods

Salmonella isolation and identification

Salmonella isolates were obtained from pastured poultry farm samples and retail carcasses. These producers had no history of antibiotic usage. Sterile sponges (drag swabs), feed, water and insect samples were obtained from two farms in Arkansas ($n = 164$). Drag swabs were rehydrated with 10 ml of 1× phosphate-buffered saline (PBS, pH 7·4). The swabs were placed in their sterile bags for transport to the laboratory. Composite feed and water samples were taken within each chicken house. For sample preparation, 10 g of feed and 10 ml of water were used for enrichment. Fly strips were suspended inside chicken houses or near chicken pens to collect insect samples for microbial analysis. Whole chicken carcasses ($n = 36$) ranging from 3 to 6 lbs (1·36–2·72 kg) were purchased from a local natural foods retail store or obtained from a local processing plant. Carcasses were rinsed for 2 min in 400 ml buffered peptone water (BPW, Becton, Dickson and Co., Sparks, MD, USA). A 50 ml aliquot of rinsate then was centrifuged at 8000 ***g*** for 5 min, and then the pellet was resuspended in 50 ml of BPW. All samples (10 g feed, 10 g water, drag swabs and carcass rinsate pellets) were pre-enriched in 50 ml of BPW for 24 h at 37°C. A 1 : 10 dilution of BPW in tetrathionate (Difco Tetrathionate broth base, Becton Dickson and Co.) and iodine solution (Ricca Chemical Company, Arlington TX, USA) was made for further enrichment and incubated another 24 h at 37°C. Tetrathionate tubes were vortexed and streaked onto XLT4 (Acumedia, Lansing, MI, USA) and Brilliant Green plus novobiocin (20 μg ml^{-1}; Becton, Dickson and Co.) agar bi-plates. Presumptive positive, sulfur-producing, black colonies were picked and passaged twice to purify them for further testing. Chrome agar (CHROMagar, Paris, France) was used to confirm *Salmonella.* To determine serotypes, antiserum (Statens Serum Institut, Copenhagen, Denmark) was used to detect O and H antigens. The Kauffmann-White scheme for *Salmonella* was used to determine serotypes (Grimont and Weill 2007).

Antimicrobial resistance evaluation

Antimicrobial susceptibility testing was conducted using the disc diffusion assay according to the CLSI (formerly NCCLS) guideline M100-S12 (NCCLS 2002). Fresh *Salmonella* cultures grown overnight on tryptic soy agar (TSA, EMD Chemicals, Gibbstown, NJ) were used. One colony from a TSA plate was suspended in 1 ml PBS (pH 7·4), and the cell suspension (OD of a 0·5 McFarland turbidity standard) was spread onto a Mueller-Hinton agar (Becton, Dickson and Co.) plate using a sterile swab (Sensititre, Trek Diagnostics Systems, Inc., Cleveland, OH, USA). Antimicrobial susceptibility test discs (Becton, Dickson and Co.) were placed on the plate and incubated at 37°C for 18–24 h. Zones of inhibition were interpreted according to the breakpoints established for Enterobacteriaceae, and isolates were identified as susceptible,

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intermediate or resistant according to the CLSI guidelines ([NCCLS] National Center for Clinical Laboratory Standards 2002). The following antimicrobials were tested: gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), novobiocin (30 μg), sulfisoxazole (250 μg), chloramphenicol (30 μg), streptomycin (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), ampicillin (10 μg) and ciprofloxacin (5 μg). *Escherichia coli* 25922 ATCC was used for quality control purposes.

Integron analysis

To isolate DNA, a loop of bacterial cells was collected from a fresh plate and suspended in 200 μl double distilled water. After vortexing, the samples were immersed in a boiling water bath for 10 min to lyse the cells, cooled at 4°C for 10 min and centrifuged at 13 000 ***g*** for 2 min. To determine the presence of integrons, a 25 μl total reaction volume, consisting of 12·5 μl Premix Ex Taq (Takara, Fisher Scientific, Pittsburgh, PA, USA), 0·5 μmol ml^{-1} of each primer, 5 μl purified DNA and water to volume was used. The integron primer sets (Int 1F, 5′-GGC ATC CAA GCA GCA AG-3′ and Int 1R, 5′-AAG CAG ACT TGA CCT GA-3′) have been previously published (Daly *et al.* 2000). Amplification was performed under the following conditions: initial denaturation of 94°C for 10 min, followed by 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. Amplicons were resolved by conventional electrophoresis through a 1% agarose gel at 80 V for 1 h. For sequencing, PCR amplicons were purified by a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and sequenced using the aforementioned primers. Sequencing was carried out in an ABI 3100 capillary analysing system (Applied Biosystems, Foster City, CA, USA), and the sequences were compared with the GenBank database using the BLAST algorithm (Altschul *et al.* 1990).

DNA fingerprints

The pulsed-field gel electrophoresis (PFGE) method was performed according to the PulseNet protocol published by the Centers for Disease Control (Ribot *et al.* 2006). The genetic relationship between the *Salmonella* strains was statistically analysed by the Bionumerics software (Applied Maths, Kortrijk, Belgium). The similarity matrix and clustering dendrogram type used for cluster analyses was calculated using the Dice band matching coefficient and the Unweighted Pair Group Method using the Arithmetic Averages (UPGMA) algorithm, respectively. A positional tolerance shift of 1·5% was permitted between similar bands and an optimization shift of 1·5% was allowed between any two patterns while generating the dendrograms.

Plasmid profiling

Plasmid DNA isolation was carried out according to the alkaline lysis mini preparation procedure outlined in Molecular Cloning 3rd Edition (Sambrook and Russel 2001). Cultures were grown in 5 ml Luria Broth (LB) (Becton, Dickson and Co.) at 37°C in a shaking water bath for 12–14 h. Alkaline lysis solution II was freshly prepared from stock solutions prior to use. The *E. coli* strain NCTC 50192, with four-well characterized plasmids (147, 63, 43·5, and 6·9 kb), were used as a size marker (Mellata *et al.* 2009). The BAC-Tracker supercoiled DNA ladder (Epicentre, Madison, WI, USA) with DNA markers from 8 to 165 kb was also used as a size marker. Plasmids were separated by PFGE on a 0·8% agarose gel for 7 h at 6 V cm^{-1} at 14°C using a CHEF-DR II electrophoresis system (Bio-Rad, Hercules, CA, USA) with an initial switch time of 1·0 and a final switch time of 6·0. Agarose gels were stained for 20 min in 0·5 μg ml^{-1} ethidium bromide and subsequently destained for 10 min in distilled water. The gel was visualized using a Gel-Doc XR system (Bio-Rad). Plasmid replicon (incompatibility) typing was carried out using the PCR-based methods described by Johnson *et al.* (2007). PCRs were separated in 2% E-gels (Invitrogen, Carlsbad, CA, USA) and compared to positive controls, graciously provided by Dr Alessandra Carattoli (Carattoli *et al.* 2005) to identify the plasmid incompatibility groups present in the strains.

Results

From a total of 200 samples from pasture farms and retail carcasses, 29·5% (n = 59/200) tested positive for *Salmonella*. These bacteria were detected in 50% of the retail carcasses (n = 36) and 25% of the farm samples (n = 164). *Salmonella* Kentucky was the prevalent serotype (53%; n = 31/59). *Salm.* Kentucky isolates were

Table 1 *Salmonella* serotypes identified among the 200 samples taken from pasture-flock poultry farms and whole carcass rinses. A total of 164 farm samples (sponges, feed, water and insect traps) and 36 whole carcasses were sampled

Serotype	Carcasses (n = 52) (% of isolates, n = 18)	Farm (n = 140) (% of isolates, n = 41)	Total (n = 200) (% of isolates, n = 59)
Salmonella Mbandaka	0 (0)	4 (10)	4 (7)
Salmonella Bareilly	0 (0)	6 (15)	6 (10)
Salmonella Kentucky	11 (61)	20 (49)	31 (53)
Salmonella Enteritidis	4 (22)	10 (24)	14 (24)
Salmonella Newport	0 (0)	1 (2)	1 (2)
Salmonella Montevideo	3 (17)	0 (0)	3 (5)
Total	18	41	59

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Table 2 Antibiotic resistance of 59 *Salmonella* isolates* obtained from free-range and pastured flock poultry farms and retail carcasses

	Ken (n = 31)		Bar (n = 6)		Ent (n = 14)		Mba (n = 4)		New (n = 1)		Mon (n = 3)	
Antibiotic	int	res	int	res	int	res	int	res	int	res	int	res
Tetracycline	0	74·2	83·3	0	28·6	0	0	100	0	0	33·3	0
Kanamycin	19·4	0	0	0	7·1	0	0	0	0	0	33·3	0
Neomycin	41·9	6·5	50	0	21·4	21·4	0	50	0	100	0	0
Sulfisoxazole	0	100	0	100	0	100	0	100	0	100	0	100
Novobiocin	0	100	0	100	0	100	0	100	0	100	0	100
Nalidixic acid	0	0	0	0	21·4	0	0	0	0	100	33·3	0
Ampicillin	0	0	0	0	14·3	0	0	0	0	0	33·3	0
Streptomycin	51·6	3·2	16·7	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0

*Serotypes: Ken, Kentucky; Bar, Bareilly; Ent, Enteritidis; Mba, Mbandaka; Mon, Montevideo; New, Newport. Percentage of isolates with int, intermediate resistance and res, high resistance were determined according to CLSI guidelines.

identified among 61% and 49% of the isolates from farm and retail carcasses, respectively (Table 1). *Salmonella* Enteritidis was the second most prevalent serotype in both sample types with 24 and 22% of farm and retail carcass isolates, respectively (Table 1). Only one isolate of *Salmonella* Newport and three of *Salmonella* Montevideo were detected. *Salmonella* Mbandaka, *Salmonella* Bareilly and *Salm.* Newport were isolated only from farm samples; however, *Salm.* Montevideo was only identified among retail isolates (Table 1).

All isolates tested were resistant to at least two antimicrobials, novobiocin and sulfisoxazole. Of the total isolates (n = 59), one isolate possessed resistance to streptomycin, 14% (n = 8/59) showed resistance to neomycin and 46% (n = 27/59) were resistant to tetracycline. Excluding novobiocin, 44% (26/59) of the *Salmonella* isolates were resistant to two antimicrobials and 10% (6/59) exhibited resistance to three; however, none of the isolates was resistant to four or more antimicrobials. Only *Salm.* Kentucky and *Salm.* Mbandaka showed resistance to tetracycline, and all *Salm.* Mbandaka isolates exhibited resistance to tetracycline. Furthermore, the only isolate to exhibit nalidixic acid resistance was *Salm.* Newport. *Salm.* Kentucky exhibited intermediate resistance to kanamycin, streptomycin and neomycin (Table 2). Only *Salm.* Enteritidis and *Salm.* Bareilly yielded intermediate resistance to nalidixic acid (Table 2). All isolates were susceptible to ciprofloxacin, gentamicin and chloramphenicol.

After PCR, integron bands were visualized through conventional gel electrophoresis in the majority of the isolates (40/59). Eight amplicon-positive samples were submitted for sequencing. The amplicon sequences (*c.* 150 bp) were compared to the NCBI Blast database. All sequences showed ≥94% similarity to a *Salmonella enterica* serotype Typhimurium class I integron. The total integron sequence length was 197 bp. Of the total isolates (n = 59), 68% were positive for this integron, while 28/41 and 12/18 isolates among the farm and retail isolates, respectively, were positive for the integron (Fig. 1). All *Salm.* Kentucky isolates were positive for the integron. Integron presence did not appear to correlate with resistance profile (Fig. 1).

The dendrogram generated through PFGE profiling revealed 19 distinct DNA profiles, consisting of 15 shared fingerprints which included farm and retail isolates and four fingerprint profiles which consisted of either farm or retail isolates (Fig. 1). Of the 19 fingerprints, 10 were found only in farm samples, five only in retail carcasess and four shared between the two sample types. *Salmonella* Mbandaka and *Salm.* Enteritidis elicited three distinct banding patterns, and two were observed for *Salm.* Bareilly (Fig. 1). *Salmonella* Kentucky exhibited the most genetic variability with nine different banding patterns. Banding patterns were not restricted to either farm or retail samples.

Five distinct plasmid profiles (PP) were observed among the isolates; *Salm.* Kentucky yielded the greatest variance. All *Salm.* Kentucky isolates harboured at least one plasmid and all but one *Salm.* Enteritidis isolate was found to carry plasmids. *Salm.* Kentucky isolates contained from one to three plasmids of approximate sizes 28, 46, 101 and/or 146 kb (Fig. 1). All *Salm.* Kentucky isolates contained the 46- kb plasmid, while *Salm.* Enteritidis isolates harboured a single, 55-kb plasmid. All but one *Salm.* Kentucky isolate was identified as positive for a single replicon incompatibility (Inc) type, either IncFIB or IncI1, regardless of the number of plasmids identified (Fig. 1). A single *Salm.* Kentucky isolate

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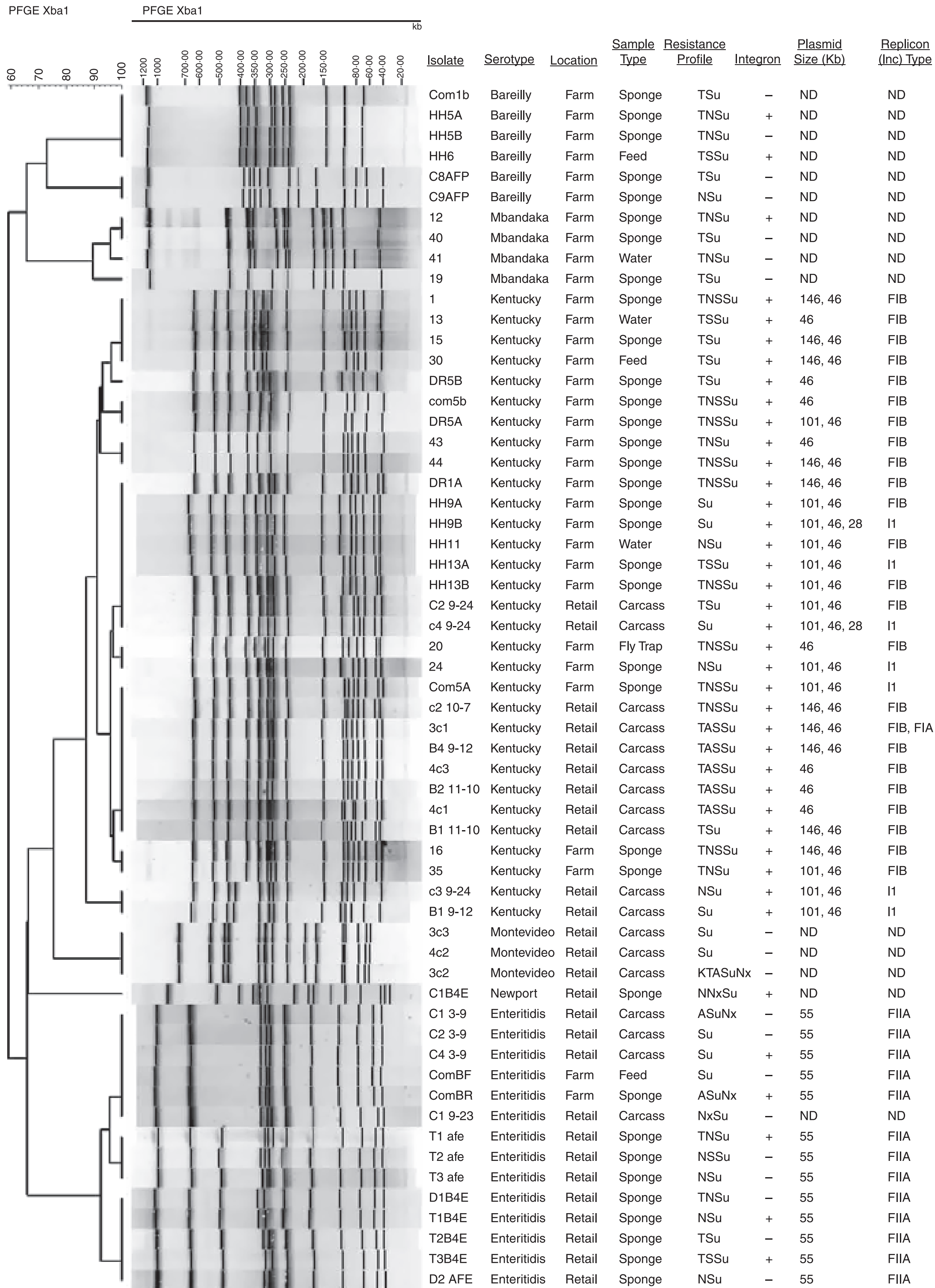

Figure 1 Dendrogram representation of *Salmonella* isolates from pastured flock poultry defined by serotype, location of origin and sample type. Additionally, antimicrobial resistance profiles (excluding novobiocin), integron presence (+/–), sizes of plasmids detected (kb) and plasmid replicon/incompatibility types detected. Antibiotic resistance was determined using the disc diffusion assay T, tetracycline; S, streptomycin; Su, sulfisoxazole; N, neomycin; Nx, nalidixic acid: integron presence was determined using conventional PCR.

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(3c1) was detected to be positive for both IncFIB and IncFIIA, while the IncIIA plasmid was identified in all but one of the *Salm.* Enteritidis isolates.

Discussion

The *Salmonella* prevalence (29·5%, 59/200) found in this study agrees with previously published prevalence data for *Salmonella* in free-range and organic poultry (Bailey and Cosby 2005; Lestari *et al.* 2009). Bailey and Cosby (2005) reported a prevalence of 31% ($n = 135$ free-range/organic) and Lestari *et al.* (2009) observed 21% *Salmonella* prevalence in organically raised poultry. In the literature, *Salmonella* prevalence rates in commercially produced chicken meat have ranged from 16 to 35%; however, much lower rates have been reported by the USDA (White *et al.* 2001; Goncagual *et al.* 2003; Jain and Chen 2006). According to the second-quarter progress report for 2009 published by the USDA, only 5·2% ($n = 2114$) of broilers tested positive for *Salmonella* (USDA, 2009).

Salmonella Kentucky has been reported as the prevalent serotype in conventional and nonconventional (free-range, pasture and organic) poultry studies (Li *et al.* 2007; Parveen *et al.* 2007; Lestari *et al.* 2009). In this study, 53% of the total isolates were identified as *Salm.* Kentucky. Here, *Salm.* Mbandaka was identified as 7% ($n = 4/59$) of pastured poultry isolates. This serotype has also been detected in 5% of isolates recovered from processed poultry (Parveen *et al.* 2007). In this study, serovar *Salm.* Enteritidis represented *c.* 24% (14/59) of the total isolates. This rate is higher than that reported by Lestari *et al.* (2009) (18·2%, $n = 11$) for organically raised birds, yet significantly lower than that reported by Marin and Lainez (2009), who reported *Salm.* Enteritidis in 67% ($n = 531$) of the samples recovered during rearing of conventional poultry. *Salm.* Enteritidis is often one of the major serotypes isolated from human and nonhuman sources and has been the second most commonly isolated serotype since 1997 (CDC 2008). According to Altekruse *et al.* (2006), although salmonellosis infections had decreased on FoodNet sites between 1990 and 2005, the incidence of *Salm.* Enteritidis was *c.* 25% higher.

Increased horizontal transmission factors, such as wildlife animal exposure, have been noted to lead to increased *Salmonella* serovar diversity in poultry flocks (Heyndrickx *et al.* 2002). However, our data indicate that the prevalence and serotype diversity of *Salmonella* in pastured flocks are similar to those reported for conventional poultry production (Li *et al.* 2007; Parveen *et al.* 2007; Lestari *et al.* 2009). While the number of samples in this study was sufficient to draw some conclusions, only two farms and one retailer were sampled. This limitation is noted because serotype prevalence is often geographically specific (Bangtrakulnonth *et al.* 2004; Galanis *et al.* 2006).

Antimicrobial resistant *Salmonella* is a great public health concern, particularly in immuno-compromised patients. Antimicrobial resistance is often attributed to the therapeutic use of antimicrobials in production systems (Jacob *et al.* 2008). Multi-drug resistant (MDR) pathogens create a hurdle in the treatment of human and animal illnesses (Daly *et al.* 2000; Jain and Chen 2006; Zhao *et al.* 2006; Maripandi 2007; Miranda *et al.* 2007, 2008). MDR strains of *Salmonella* have been linked to increased morbidity, compared to susceptible strains (Daly *et al.* 2000). In this study, all of the isolates were resistant to at least one antimicrobial. However, excluding novobiocin, none of the isolates from pasture poultry were resistant to five or more antimicrobials. In *Salmonella* from conventional poultry, Siemon *et al.* (2007) and Cui *et al.* (2005) reported 69 and 64% of isolates as resistant to five or more antimicrobials.

Studies have expressed increased susceptibility of nonconventional poultry isolates to *Salmonella*, compared to conventional poultry isolates (Siemon *et al.* 2007; Miranda *et al.* 2008). Cui *et al.* (2005) reported that, in *Salmonella* Typhimurium, tetracycline resistance increased by 15% from 1980 to 1990 in the United States. Of the antimicrobials tested in this study, 48% (28/59) were resistant to tetracycline. Parveen *et al.* (2007) reported that 73% of *Salmonella* from commercially processed conventionally raised poultry were resistant to tetracycline. In particularly, the same study reported nearly all the *Salm.* Kentucky isolates were resistant to tetracycline and beta-lactams which is similar to our findings.

In this study, PCR primers targeting class I integrons detected in 68% (40/59) of *Salmonella* isolates from pastured-reared poultry. In 2002, Liebana *et al.* determined that 6·9% ($n = 100$) of nontyphoidal *Salmonella* isolates from commercial farm animals contained class I integrons. Furthermore, Martin *et al.* (2008) found that 29% ($n = 35$) *Salmonella* isolates from swine possessed class I integrons. To the best of our knowledge, this study is the first report of class I integron prevalence among *Salmonella* isolates from pastured poultry. The high frequency for integrons among the isolates here may be because of the low number of sampled sources; however, it may also be a reflection of the pasture system with increased contact with the environment.

Five PP were observed, with relative plasmid sizes of 28, 46, 55, 101 and 146 kb (Fig. 1). Some of the plasmid sizes detected among the *Salm.* Kentucky isolates here are similar to those reported by Fricke *et al.* (2009), who determined the plasmid sizes of poultry-derived *Salm.* Kentucky isolates to be 146 811, 101 461 and 46 121 bp. Of the 31

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Salm. Kentucky isolates in this study, 74% contained large (>100 kb) plasmids, which are often associated with antimicrobial resistance (Kaldhone *et al.* 2008). For all but one *Salm.* Kentucky isolate, a single Inc group was identified, even though isolates were identified as having as many as three plasmids. The current plasmid replicon typing schemes are limited in that not all replicon types are represented and thus some of the plasmids were not characterized by the typing. For example, the 46- kb plasmid described by Fricke *et al.* (2009) (GenBank accession number CP001123) does not have sequence similarity to the replicon typing primers used in this study. The two predominant plasmid Inc groups (FIB and I1) contained plasmids greater than 100 kb in size and carried antimicrobial resistance genes (Johnson *et al.* 2006; García-Fernández *et al.* 2008; Fricke *et al.* 2009). All the isolates identified as containing IncFIB plasmids were resistant to tetracycline, regardless of whether they contained a 101- or 146- kb large plasmid. Conversely, all but one IncI1 positive isolates was susceptible to tetracycline and each contained a 101- kb plasmid. Eight isolates were detected to contain IncFIB plasmids, yet did not have a large plasmid. Each of these isolates was multidrug resistant, including to tetracycline, which suggests that the isolates contain an additional large plasmid that was not isolated using the standard methods.

The *Salm.* Enteritidis plasmid of size 55 kb is commonly found among the majority of *Salm.* Enteritidis isolates and has been denoted the serovar-specific plasmid (Bakeri *et al.* 2003). The replicon typing results are in agreement with these finding, because the common *Salm.* Enteritidis virulence plasmids are known to be IncFIIA plasmids (Carattoli *et al.* 2005; Hong *et al.* 2008). Of their chicken-derived *Salm.* Enteritidis isolates, Icgen *et al.* (2002) found that over 90% contained a plasmid of size 54 kb. Furthermore, Poppe *et al.* (1995) found 97% of poultry-derived *Salm.* Enteritidis isolates to contain a plasmid of 36 MDa (*c.* 55 kb). Only one plasmid profile was observed from *Salm.* Enteritidis in this study. Rodrigue *et al.* (1992) expressed that differences in PP may reflect a difference in poultry production system or lack of antibiotic use, which may also contribute to the lack of profiles found in this study.

The *Salmonella* PFGE fingerprints were separated into 19 genetically distinct groups based on a 90% or higher similarity cut-off (Fig. 1). The close genetic relationship among the isolates of serotype *Salm.* Enteritidis genotypes aligns with the findings of Valdezate *et al.* (2007), who noted a lack of genetic diversity in this serotype. Interestingly, the PFGE results often indicated genotypic similarity between samples isolated from distinct locations on a farm and even among carcass and farm isolates. For example, one of the *Salm.* Enteritidis genotypic groups contains isolates from sponge, feed and carcass samples (Isolates ComBR, ComBF, and C1 9-23, respectively). This may suggest that there is a degree of cross-contamination on the farm. Cross-contamination is a food safety concern, especially in broiler flocks, because subsequent contamination of the processing plant may occur. Olsen *et al.* (2003) found that poultry from two of eight negative flocks tested positive for *Salmonella* after processing, and five of ten positive flocks had increased serotype diversity after processing.

Salmonella isolates from the pastured poultry farms and purchased poultry carcasses in this initial survey exhibited similar antimicrobial resistance and possessed class I integrons. The prevalence and serotypes identified from these pastured poultry are similar to those reported by conventional poultry studies. However, more farms should be surveyed to draw more definitive conclusions.

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