Screening of *Salmonella* Isolates from a Turkey Production Facility for Antibiotic Resistance

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**ABSTRACT** An ecological survey was conducted from April 1997 to June 1999 on four turkey flocks (F1 to F4). Turkey cecal contents, litter, waterers, feed, feeders, and environmental swabs were analyzed. Presence of *Salmonella* was determined using conventional microbiological screening techniques and confirmed by serology. Positive isolates were serotyped and screened for antibiotic resistance. From a total of 69 *Salmonella* isolates 25% were resistant to one or more antibiotics including gentamicin (G), spectinomycin (SP), streptomycin (S), tetracycline (T), tobramycin (TO), and trimethoprim/sulfamethoxazole. Isolates included 45 *S. heidelberg*, 13 *S. senftenberg*, 7 *S. muenster*, 2 *S. anatum*, and 2 *S. worthington*. Resistance to antibiotic(s) was highest among waterer isolates (55%) followed by environmental swabs (43%), feeder content samples (33%), turkey cecal contents (26%), and litter samples (5%). Frequencies of antibiotic-resistant *Salmonella* in F1, F2, and F4 were 27, 13, and 40%, respectively. *Salmonella* was undetected in F3. In F1, *S. heidelberg* from cecal content and waterer samples was resistant to G, SP, S, and T, whereas *S. anatum* from waterer samples was resistant to T and S. In F2, *S. muenster* from litter and feeder content samples was resistant to T, and in F4, *S. muenster* from environmental swabs was resistant to TO, S, SP, and G. Identifying preharvest sources and characterizing serotype and antibiotic-resistance profile can assist poultry producers and integrators in tracking movement of *Salmonella* on turkey farms.

(Key words: *Salmonella*, turkey production, antibiotic resistance, preharvest sources)

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INTRODUCTION

Antibiotics are added to poultry feed and water at low levels (15 to 25 ppm) to improve bird performance and at high levels (100 to 200 ppm) to treat specific bacterial diseases (Stavric and D’Aoust, 1993). Widespread use of antibiotics in food animals has resulted in emergence of antibiotic-resistant strains of food borne pathogens, such as *Salmonella*, *Escherichia coli*, and *Campylobacter*, as well as bacteria endogenous to the microflora of animals (Davis et al., 1999; van den Bogaard and Stobberingh, 1999). These resistant strains may be transmitted to humans through food. Multiple drug-resistant isolates account for 20 to 25% of human *Salmonella* infections in the US (Holmberg et al., 1984). Several strains of multiple-drug-resistant *Salmonella* strains have been isolated from farm animals and foods of animal origin (DuPont and Steele, 1987; Threlfall et al., 1993; Ansari and Khatoon, 1994; Nair et al., 1995; Aarestrup et al., 1998; Tollefson et al., 1998; Klien, 1999; van der Wolf et al., 1999; Wagner and Hahn, 1999). Every tenth nontyphoidal *Salmonella* isolate received by the state public health laboratories in the US from July 1994 to June 1995 was resistant to 12 antimicrobial agents (Herikstad et al., 1997).

*Salmonella* and other food borne pathogens acquire antibiotic resistance by random chromosomal mutations, mutation of existing genes, and through specific mechanisms such as transduction, tranformation, and conjugation (Okolo, 1986). These mechanisms involve transfer of drug-resistant genes by means of circular DNA plasmids such as R-factor, conjugative plasmid, or chromosomal elements (Vidon et al., 1978; O’Brien et al., 1982; Holmberg et al., 1984; Poppe and Gyles, 1987; Schuman et al., 1989; Salyers and Whitt, 1994; Poppe et al., 1996; Wagner and Hahn, 1999). One such strain, *S. typhimurium* DT 104, is resistant

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Abbreviation Key: BHI = brain heart infusion; F1 = flock 1; F2 = flock 2; F3 = flock 3; F4 = flock 4; G = gentamicin; LIA = lysine iron agar; SP = spectinomycin; S = streptomycin; T = tetracycline; TO = tobramycin; TSI = triple sugar iron; UP = universal pre-enrichment.
to antibiotics such as ampicillin, chloramphenicol, streptomycin (S), sulfonamides, tetracycline (T), and trimethoprim (Herikstad et al., 1997). Approval of drugs such as sarafloxacin in poultry has contributed to the emergence and spread of *Salmonella* strains resistant to fluoroquinolone (nalidixic acid and ciprofloxacin) (Herikstad et al., 1997). Human infections with DT 104 are associated with consumption of contaminated meat and meat products, and the pathogen has been traced back to infected farm animals. Manie et al. (1998) found several strains of multi-resistant *Salmonella* collected from turkeys and environmental sources in the turkey production facility.

**MATERIALS AND METHODS**

A survey was conducted at Wardensville, WV. This facility contained 12 pens on either side of a centrally located service area, for a total of 24 pens. Four consecutive turkey flocks (F1 to F4) were sampled in this facility from April 1997 to June 1999.

**Sampling Procedures**

Litter, waterer, air, and feed samples, and environmental swabs were collected from each flock throughout the grow-out period (Table 1). Litter, waterer, and turkey sampling was synchronized so that positive samples could be identified by the pen source. Poults entering the facility and box liners in which poults were shipped were sampled for an indication of *Salmonella* contamination from the breeder flocks or the hatchery. Liners were sampled only for F3. Before placement of the poults in F3 (Day 0), litter, waterers, air, feed, and environmental swab samples were collected from various locations in the facility to determine the initial level of contamination. In F3, 12 poults from each of three hatcheries, for a total of 36 poults, were euthanized by cervical disarticulation (American Veterinary Medical Association, 1993) at Day 0, and the entire intestine and yolk sac were transferred to 100 mL of universal preenrichment (UP) broth. At Week 2, intestinal samples were transferred to 100 mL of UP broth. From Week 10 until the end of the grow-out period, the entire ceca were removed, the blind end was snipped with sterile scissors, and cecal contents were emptied into sterile stomacher bags and sealed. Crop contents were sampled in F3 and F4 by removing the crop, making an incision with a sterile scalpel, and transferring the contents into a sterile stomacher bag. Litter samples were collected from the top 5.08-cm layer of litter and placed in sterile bags. These bags were held on ice during transport to the laboratory. Waterers were swabbed with sterile cloth gauze (25 cm²) held by a pair of sterilized forceps. Environmental swabs were collected from various surfaces in the facility with a 16-cm² template using a sterile swab moistened with sterile broth. Locations included walls, ventilation fans, feathers of dead birds, employee shoes, the feed truck, fans inside the pens, feed storage bins, and door handles. Swabs were transferred to 10 mL sterile UP broth. Air samples were collected on Rodac plates (65 × 15 mm) containing brain heart infusion (BHI) agar using a SAS portable high flow air-sampler. The air sampler was set to collect 60 L of air in 20 s. The agar was aseptically transferred to sterile stomacher bags. Feed and feeder samples were collected randomly from each feed shipment by placing a sterile bag in the flow of feed from the auger or from the feed cart. Samples were stored at −4 C at the production facility prior to transport to the laboratory for analyses. All samples were held on ice during transport to the laboratory. Transport time did not exceed 4 h.

**Laboratory Procedures**

Samples were mixed with UP broth (1:10 wt/vol) and sealed. Gauze and agar from waterer and air samples, respectively, were mixed with 100 mL UP broth. Approximately 5 g of litter and 10 g of feed or feeder content samples, respectively, were transferred to bottles containing 100 mL UP broth. Samples were thoroughly mixed with UP broth and incubated for 24 h at 37 C. One milliliter of this pre-enriched sample was transferred to 9 mL of tetrahionate broth and incubated for 24 h at 37 C. Selectively enriched samples from tetrahionate broth were streaked to isolation on xylose lactose tergitol (XLT4) plates. These plates were incubated at 37 C for 24 h. A single presumed positive *Salmonella* colony was stabbed and streaked on triple sugar iron (TSI) and lysine iron

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1 Figures in parentheses indicate percentage positive.
were resistant to T and S. Of the six S. heidelberg (Table 2). Two isolates of 27% of serotypes were resistant to two or more antibiotics 5% of litter samples (Table 1). Of the 48 isolates in F1, swabs, 33% of feeder contents, 26% of turkey ceca, and were antibiotic resistant were 55% of waterers, 43% of on source, in F1, F2, and F4, the percentage of isolates that resistant to S, SP, and G, whereas the remaining isolate collected from turkey ceca at Week 18, five isolates were of S. heidelberg S. anatum S. muenster was the most prevalent serotype, accounting for 65% of the isolates from these flocks. Other serotypes were identified as S. anatum (10%), S. muenster (29%), and S. worthington (3%). Of the 48 isolates serotyped in F1, 92% were S. heidelberg, 4% S. muenster, and 4% S. anatum (Nayak, 2000).

RESULTS AND DISCUSSION

Sixty-nine isolates were serotyped from 58 Salmonella-positive samples in F1, F2, and F4. Salmonella was not detected in F3. Salmonella heidelberg was the most prevalent serotype, accounting for 65% of the isolates from these flocks. Other serotypes were identified as S. senftenberg (19%), S. muenster (10%), S. anatum (3%), and S. worthington (3%). Of the 48 isolates serotyped in F1, 92% were S. heidelberg, 4% S. muenster, and 4% S. anatum (Nayak, 2000).

Of the 69 Salmonella serotypes screened, 25% were resistant to one or more of the following antibiotics: G, spectinomycin (SP), S, T, tobramycin (TO), and trimethoprim / sulfamethoxazole. Among the isolates screened, 100% of S. anatum and S. worthington, 29% of S. muenster, and 17% of S. heidelberg were resistant to the above antibiotics. Based on source, in F1, F2, and F4, the percentage of isolates that were antibiotic resistant were 55% of waterers, 43% of swabs, 33% of feeder contents, 26% of turkey ceca, and 5% of litter samples (Table 1). Of the 48 isolates in F1, 27% of serotypes were resistant to two or more antibiotics (Table 2). Two isolates of S. anatum from waterers at Week 2 were resistant to T and S. Of the six S. heidelberg isolates collected from turkey ceca at Week 18, five isolates were resistant to S, SP, and G, whereas the remaining isolate was resistant to T, S, SP, and G. Two isolates of S. heidelberg collected from waterers at Week 18 were resistant to S, SP, and G and the remaining two were resistant to T, S, SP, and G. One isolate of S. heidelberg from an environmental swab taken at Week 18 was resistant to T and trimethoprim/sulfamethoxazole. Antibiotic-resistant strains of S. heidelberg isolated from waterer samples at Week 18 might have been horizontally transmitted by turkeys.

Of the 16 isolates in F2, 13% were resistant to T (Table 3). At Week 21 of the grow-out period S. worthington, detected in litter and feeder content samples, was resistant to T. Of the five isolates in F4, two were resistant to TO, S, SP, and G (Table 4). These isolates were S. muenster, taken from environmental swabs.

Antimicrobial agents are fed at subtherapeutic levels to poultry to enhance growth rate and feed efficiency, and at therapeutic levels to prevent bacterial infections. Polymyxin B and combinations of 1) trimethoprim and sulfadiazine, 2) neomycin and polymyxin, and 3) trimethoprim and polymyxin B have been administered in feed or drinking water to reduce the levels of Salmonella in chickens (Craven, 1995). Williams (1985) reported that feeding oxytetracycline or neomycin, or both reduced S. typhimurium levels in broiler intestines. On the other hand, feeding antibiotics such as avoparicin and lincomycin favored colonization of S. typhimurium, whereas nitrofurazone enhanced colonization of S. infantis (Glisson, 1998). Spread of Salmonella to contact chickens was subsequently observed. The increase in Salmonella colonization could have resulted from decreased natural gut microflora, therefore, less competition.

Antibiotics, by a process of selection, can facilitate the proliferation of resistant population of microorganisms. Gast et al. (1988) reported 40% isolation of drug-resistant S. typhimurium from kanamycin-treated rats fed a diet containing liver from kanamycin-treated poults. In another

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serotype</th>
<th>Frequency</th>
<th>Serotype</th>
<th>Frequency</th>
<th>Serotype</th>
<th>Frequency</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey ceca</td>
<td>S. heidelberg</td>
<td>0/6</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
<td>6/15</td>
<td>6/22 (27%)^4</td>
</tr>
<tr>
<td>Litter</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
<td>0/2</td>
<td>S. heidelberg</td>
<td>0/6</td>
<td>0/10</td>
</tr>
<tr>
<td>Waterers</td>
<td>S. anatum</td>
<td>2/2</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
<td>4/7</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Environmental swabs</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
<td>1/4</td>
<td></td>
<td></td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2/11</td>
<td></td>
<td>0/5</td>
<td></td>
<td>11/32</td>
<td>13/48 (27%)</td>
</tr>
</tbody>
</table>

^1T = tetracycline; S = streptomycin; SP = spectinomycin; G = gentamicin; TS = trimethoprim / sulfamethoxazole.

^2Antibiotic resistant isolates per total isolates.

^3Number of isolates and resistant antibiotics.

^4Figures in parentheses indicate percentage positive.

TABLE 2. Antibiotic resistance among Salmonella serotypes isolated from Flock 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Week 2</th>
<th>Week 10</th>
<th>Week 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Frequency</td>
<td>Serotype</td>
<td>Frequency</td>
</tr>
<tr>
<td>Turkey ceca</td>
<td>S. heidelberg</td>
<td>0/6</td>
<td>S. heidelberg</td>
</tr>
<tr>
<td>Litter</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
</tr>
<tr>
<td>Waterers</td>
<td>S. anatum</td>
<td>2/2</td>
<td>S. heidelberg</td>
</tr>
<tr>
<td>Environmental swabs</td>
<td>S. heidelberg</td>
<td>0/1</td>
<td>S. heidelberg</td>
</tr>
</tbody>
</table>
TABLE 3. Antibiotic<sup>1</sup> resistance among *Salmonella* serotypes isolated from Flock 2

<table>
<thead>
<tr>
<th>Grow-out period</th>
<th>Week 13</th>
<th>Week 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serotype</td>
<td>Frequency</td>
</tr>
<tr>
<td>Turkey ceca</td>
<td>. . . .</td>
<td>. . . .</td>
</tr>
<tr>
<td>Litter</td>
<td>S. senftenberg</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>S. heidelberg</td>
<td>0/1</td>
</tr>
<tr>
<td>Feed</td>
<td>S. senftenberg</td>
<td>0/2</td>
</tr>
<tr>
<td>Feeder contents</td>
<td>. . . .</td>
<td>. . . .</td>
</tr>
<tr>
<td>Total</td>
<td>0/11</td>
<td>2/5 (40%)</td>
</tr>
</tbody>
</table>

<sup>1</sup>T = tetracycline.

<sup>2</sup>Antibiotic resistant isolates per total isolates.

<sup>3</sup>Number of isolates and resistant antibiotic.

<sup>4</sup>Figures in parentheses indicate percentage positive.

A survey component of this study found that low frequency of *Salmonella* detection in F2, F3, and F4 was attributed to the use of Termin-8, an antimicrobial feed additive used to inhibit *Salmonella* (Nayak, 2000). Therefore, based on the limitation of low levels of *Salmonella* detection, the current study does not infer antibiotic resistance associated with a specific source. Nonetheless, on the average approximately 25% of *Salmonella* serotypes isolated were antibiotic resistant, and throughout the study *S. heidelberg* was identified most often for antibiotic resistance. Identifying preharvest sources, characterizing serotypes, and determining antibiotic-resistance profiles can assist poultry producers and integrators in tracking *Salmonella* transmission pathways and in planning antibiotic use strategies.

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