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# Dissemination of Fluoroquinolone-Resistant *Campylobacter* spp. within an Integrated Commercial Poultry Production System

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**While characterizing the intestinal bacterial community of broiler chickens, we detected ε-proteobacterial DNA in the ilea of 3-day-old commercial broiler chicks (J. Lu, U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee, Appl. Environ. Microbiol. 69:6816–6824, 2003). The sequences exhibited high levels of similarity to *Campylobacter jejuni* and *Campylobacter coli* sequences, suggesting that chickens can carry *Campylobacter* at a very young age. *Campylobacter* sp. was detected by PCR in all samples collected from the ilea of chicks that were 3 to 49 days old; however, it was detected only in the cecal contents of chickens that were at least 21 days old. In order to determine whether the presence of *Campylobacter* DNA in young chicks was due to ingestion of the bacteria in food or water, we obtained commercial broiler hatching eggs, which were incubated in a research facility until the chicks hatched. DNA sequencing of the amplicons resulting from *Campylobacter*-specific 16S PCR performed with the ileal, cecal, and yolk contents of the day-of-hatching chicks revealed that *Campylobacter* DNA was present before the chicks consumed food or water. The 16S rRNA sequences exhibited 99% similarity to *C. jejuni* and *C. coli* sequences and 95 to 98% similarity to sequences of other thermophilic *Campylobacter* species, such as *C. lari* and *C. upsaliensis*. The presence of *C. coli* DNA was detected by specific PCR in the samples from chicks obtained from a commercial hatchery; however, no *Campylobacter* was detected by culturing. In order to determine whether the same strains of bacteria were present in multiple levels of the integrator, we cultured *Campylobacter* sp. from a flock of broiler breeders and their 6-week-old progeny that resided on a commercial broiler farm. The broiler breeders had been given fluoroquinolone antibiotics, and we sought to determine whether the same fluoroquinolone-resistant strain was present in their progeny. The isolates were typed by pulsed-field gel electrophoresis, which confirmed that the parental and progeny flocks contained the same strain of fluoroquinolone-resistant *C. coli*. These data indicate that resistant *C. coli* can be present in multiple levels of an integrated poultry system and demonstrated that molecular techniques or more sensitive culture methods may be necessary to detect early colonization by *Campylobacter* in broiler chicks.**

*Campylobacter jejuni* and *C. coli* have been recognized as major causes of sporadic food-borne enteritis in humans (40). Recently, workers have focused on this pathogen because of the emergence of antibiotic-resistant *Campylobacter* isolates that cause disease in humans (38, 42). The Centers for Disease Control and Prevention have estimated that 2 million persons were infected with *Campylobacter* in the United States from 1996 to 1999 (33). Food-borne illnesses are a major public health concern, and many of these illnesses may be linked to the consumption of poultry products. Raw poultry meat is considered to be an important vector of *Campylobacter* (13, 33). Some reports indicate that 80% of broiler chickens that are between 5 and 7 weeks old can carry *C. jejuni* or *C. coli* in the intestinal tract (29, 32).

Many studies have demonstrated that horizontal transmission is the major route of *Campylobacter* colonization of poultry because the same strain can be shown to colonize multiple flocks on a farm (29, 32). However, some investigations have detected egg-associated bacteria, suggesting that vertical transmission could occur (1, 6, 11, 27, 36). Fertile chicken eggs can be infected experimentally with *C. jejuni* (6), and the organism can be recovered from the inner membranes of the eggs and egg contents (35). Chuma et al. detected *C. jejuni* in the cecal contents of newly hatched chicks using DNA-DNA hybridization (5). Several recent reports have shown that *C. jejuni* can colonize the oviducts of laying hens and turkeys (3, 4, 7, 11, 17). *Campylobacter* spp. have also been detected in semen samples of commercial broiler breeder roosters and turkey toms, which provides an opportunity for venereal infection of hens (7, 10, 18).

In a characterization of the bacterial community of the broiler chicken intestine, we detected ε-proteobacterial 16S rRNA sequences in the ilea of 3-day-old chicks (23). The majority of the clones in the intestinal 16S rRNA library were most similar to the sequence of *C. coli* 16S rRNA. In the current study, we detected the same strain of fluoroquinolone-resistant *C. coli* in a flock of commercial broiler breeders and their progeny and obtained molecular evidence that *Campylobacter* can be present in day-of-hatching chicks.

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## MATERIALS AND METHODS

**Sample collection.** Freshly voided cecal droppings were collected from a flock house (~22,000 Ross/Cobb hybrid chickens per house) on each of three com-

mercial broiler farms (farms CF-1, CF-2, and CF-3); two flocks were sampled from each farm. These poultry farms had contracted to raise broiler chickens for the same poultry company. Most houses were sampled when the birds were 3 and 6 weeks old; however, flock 1 on farm CF-2 was also sampled when the chickens were 5 and 7 weeks old, before and after oxytetracycline treatment at 6.5 weeks. In addition, the birds on farm CF-3 were given sarafloxacin when they were 5 weeks old; the approved dosage was 20 ppm in the drinking water for 5 days. Farms CF-2 and CF-3 had an antibiotic usage history of sarafloxacin and oxytetracycline administration in the drinking water during the year before sampling, and farm CF-1 had not used therapeutic antibiotics for more than 1 year. For each sampling, approximately 120 cecal droppings were collected from the top of the chicken litter using sterile cotton-tipped swabs. The 120 swabs were pooled in 30 tubes, each of which contained 1 ml of brain heart infusion broth, and were placed on ice until they were cultured.

Through information obtained by our clinicians at the Poultry Diagnostic and Research Center, we located a commercial broiler breeder (parental) flock that had been treated with a poultry fluoroquinolone (Saraflox; Abbott Laboratories, Abbott Park, IL). The broiler breeder farm had contracted with the same poultry company as our three broiler chicken farms. One week after antibiotic administration, we collected 120 cecal droppings from this flock. Cecal droppings were also collected from a flock of broilers that were hatched from eggs from the breeders. Samples were placed in brain heart infusion broth, cooled on ice, and transported to the laboratory for culture.

Ten embryonating eggs were obtained from the treated breeder flock and incubated in a research hatching cabinet until the chicks emerged. Ten chicks from the same breeder flock were also obtained from the commercial broiler hatchery before they were placed on a farm. None of the chicks had access to food or water before they were sacrificed. The chicks were sacrificed on the day of hatching by $CO_2$ asphyxiation. The surface of the necropsy table and the chick surfaces were disinfected with 70% ethanol, and the ileum, cecum, and yolk sacs were aseptically removed using sterile instruments. A separate set of sterile instruments was used for each chick. Ileum, cecum, and yolk contents from each set of chicks were pooled in sterile tubes for culture and DNA extraction.

***Campylobacter* culture.** Culturing from the cecal droppings of commercial broiler flocks and the broiler breeder flock was performed as follows. The contents of the 30 tubes containing pooled cecal droppings were pooled into 10 tubes and diluted with saline. *Campylobacter* spp. were isolated from $10^{-1}$ and $10^{-2}$ dilutions by membrane filtration (12) by placing sterile cellulose acetate membrane filters (pore size, 0.45 μm; diameter, 25 mm) on the surfaces of blood agar plates. One hundred microliters of a diluted sample was carefully placed on top of a filter, avoiding spillage around the edges of the filter, and the plates were incubated at room temperature for 30 min until the liquid was absorbed by the agar. The filters were removed using sterile tweezers, and the plates were placed in Ziploc bags that were flushed with a microaerophilic gas mixture (10% $CO_2$, 5% $O_2$, 85% $N_2$). The plates were incubated at 37°C and observed for 72 h for growth. Thirty isolated *Campylobacter*-like colonies (gray, watery) were selected in order to detect resistant phenotypes with a flock prevalence of 5% or greater. The colonies were randomly selected from the 10 plates, streaked for isolation, placed in freezer medium (15% glycerol, 1% peptone), and stored at −80°C. However, in order to enhance detection of a resistant strain of *C. coli* in the breeder flock, blood agar containing 1 μg/ml of ciprofloxacin was used to culture *Campylobacter* from the broiler flock.

Pooled samples from chick ileum, cecum, and yolk contents were directly plated on blood agar using the direct filtration method described above in order to avoid the use of selective agar media that may inhibit some *Campylobacter* species or isolates (12, 37). In addition, *Campylobacter* culturing from the pooled samples obtained from day-of-hatching chicks was done using enrichment broth containing 0.6% yeast extract or tryptic soy broth containing 0.6% yeast extract as described by Moore (25). Duplicate enrichment tubes were incubated in one of two microaerophilic gas mixtures as described by Engberg et al. (12); one of these gas mixtures was hydrogen enriched (6% $CO_2$, 6% $O_2$, 85%$N_2$, 3% $H_2$), and the other was not (10% $CO_2$, 5% $O_2$, 85% $N_2$). The enrichment broth preparations were loosely capped, placed in a rack that was placed in a Ziploc bag, flushed with gas, and incubated with gentle shaking at 37°C. After incubation for 24 h, 30 μl from each tube was streaked on blood agar plates.

Susceptibility testing for *C. jejuni* was performed by the agar dilution method as described by the National Committee for Clinical Laboratory Standards (26), using *C. jejuni* ATCC 33560 as the quality control organism.

**Molecular detection of *Campylobacter*.** Detection of *C. jejuni* or *C. coli* in the *Campylobacter* isolates cultured from the broiler breeder and commercial broiler flocks was performed by species-specific PCR as described by Gonzales et al. (14). Templates from the isolates were prepared as described by Woods et al. (43). PCR detection of *Campylobacter* in community DNA samples acquired from a previous study (23) was performed using *Campylobacter*-specific 16S rRNA primers (22). PCR detection of *Campylobacter* in day-of-hatching chick ileum, cecum, and yolk samples was performed using *Campylobacter*-specific 16S rRNA primers (22) or species-specific *Campylobacter* primers (14). *C. jejuni* ATCC 33560 and a chicken isolate of *C. coli* (M1-19), whose identity was confirmed by biochemical testing, were used as positive controls. The template from day-of-hatching chicks was isolated as follows. The bacterial fraction was recovered from the enrichment broth by centrifugation (5,000 × *g* for 6 min) at room temperature. DNA was extracted using a Mo Bio kit (Mo Bio Laboratories Inc., California). In order to determine the distribution of *Campylobacter* species in the chick ileum, cecum, and yolk samples, the *Campylobacter*-specific 16S rRNA amplicons were cloned and the DNA was sequenced as previously described (23). The resulting sequences were analyzed by BLAST algorithms (www.ncbi.nlm.nih.gov) and were used to search the GenBank database in order to determine similarities to known species of bacteria. Phylogenetic relatedness among 16S rRNA sequences was evaluated by neighbor-joining analysis of a distance matrix obtained from a multiple-sequence alignment.

**Molecular typing.** The *Campylobacter* isolates were initially typed by the randomly amplified polymorphic DNA (RAPD) method, using primer HWL85 as described by Zimmer et al. (44). Isolates from the broiler breeder flock and commercial flock that produced the same RAPD pattern were further typed using pulsed-field gel electrophoresis (PFGE). PFGE was performed as follows. Isolates were grown as confluent lawns on blood agar under microaerophilic conditions. Bacterial cells were then harvested by scraping each plate with an inoculating loop and suspended in 2 ml of phosphate-buffered saline. Cells were pelleted by centrifugation at 18,000 × *g* for 2 min and then suspended in 1.2 ml PIV buffer (10 mM Tris [pH 7.4], 1 M NaCl). Subsequent steps were performed as described by Barrett et al. (2). The agarose-embedded bacterial genomic DNA was digested with 30 U of SmaI or KpnI by incubation overnight at 25°C for SmaI and at 37°C for KpnI. DNA fragments were separated by PFGE in a 1.2% agarose gel using a CHEF DR-II electrophoresis unit (Bio-Rad, Hercules, California). Electrophoresis was performed for 25 h at 14°C and 200 V with a linearly ramped pulse time of 5 to 30 s. *Saccharomyces cerevisiae* DNA was used as the molecular weight marker (Bio Whittaker Molecular Applications, Rockland, ME).

**Nucleotide sequence accession numbers.** Representative sequences have been deposited in the GenBank database under accession numbers DQ057348 to DQ057352.

## RESULTS

**Detection of fluoroquinolone-resistant *Campylobacter* from untreated commercial broiler flocks.** Table 1 shows the distribution of ciprofloxacin susceptibility among *Campylobacter* isolates cultured from commercial broiler chickens. Sixty percent of the *C. jejuni* isolates and 23.5% of the *C. coli* isolates exhibited MICs of ciprofloxacin of ≥4 μg/ml. Since ciprofloxacin breakpoints have not been established for *Campylobacter* species, we utilized the ≥4-μg/ml level used by the National Antimicrobial Resistance Monitoring System (http://ars.usda.gov/SP2UserFiles/Place/66120508/NARMS/animal_campy/campy2003histogram.pdf; accessed 9 May 2005) to categorize *Campylobacter* isolates as resistant. Ciprofloxacin-resistant *Campylobacter* isolates were detected in four of the six flocks, although only one flock had been treated with a fluoroquinolone. Two of the farms had a recent history of fluoroquinolone usage, but on farm CF-1, from which we isolated ciprofloxacin-resistant *C. jejuni* and *C. coli*, therapeutic antibiotics had not been used within 1 year of our sampling and fluoroquinolones had not been used in the flock house that was sampled. However, all of the farms had contracts with the same poultry integrator, suggesting that the ciprofloxacin-resistant *Campylobacter* isolates could have originated from a common source. In order to investigate whether a common *Campylobacter* strain was present on all three broiler chicken farms, isolates from each sampling were typed using RAPD-PCR. Several *Campylobacter* isolates from different farms produced similar RAPD fingerprints (data not shown), and

TABLE 1. Fluoroquinolone susceptibility ($MIC_{90}$) of *C. jejuni* and *C. coli* cultured from commercial broiler chicken flocks at various ages[a]

| Farm[b] | Ciprofloxacin $MIC_{90}$ (μg/ml) (no. of isolates) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Flock 1 | | | | Flock 2 | | | |
| | 3–5 wks | | 6–7 wks | | 3 wks | | 6 wks | |
| | *C. jejuni* | *C. coli* | *C. jejuni* | *C. coli* | *C. jejuni* | *C. coli* | *C. jejuni* | *C. coli* |
| CF-1 | ND[c] | ND | 8 (24) | 8 (2) | 0.25 (30) | ND | 2 (25) | 2 (5) |
| CF-2 | 0.25 (30) | ND | 0.5 (58) | 0.5 (2) | 16 (21) | 16 (9) | 16 (30) | ND |
| CF-3 | ND | ND | 4 (7)[d] | 16 (2)[d] | 0.125 (22) | 8 (8) | 16 (26) | 16 (4) |

[a] *C. jejuni* (273 isolates) and *C. coli* (31 isolates) were cultured from the fresh cecal droppings of two consecutive flocks of commercial broiler chickens located on three geographically separated farms. Fluoroquinolone susceptibility was determined by agar dilution. All of the isolates from the same sampling exhibited the same MIC.

[b] *Campylobacter* was cultured from most flocks when the birds were 3 and 6 weeks old; the exception was flock 1 on farm CF-2, from which *Campylobacter* was cultured when the birds were 5, 6, and 7 weeks old.

[c] ND, no bacteria detected by culturing.

[d] The birds were given sarafloxacin (20 ppm for 5 days) in their drinking water when they were 5 weeks old. *Campylobacter* isolates were cultured after this treatment.

these isolates were compared further using PFGE. Figure 1A shows the results of the PFGE typing for the *Campylobacter* isolates cultured from the second flock from farms CF-1 and CF-3. These *Campylobacter* isolates produced the same SmaI PFGE pattern, indicating that the same strain may be present on multiple broiler chicken farms. This finding suggests that the *Campylobacter* strain present on these broiler chicken farms may have come from a common source at the hatchery or in the breeder flock.

**Detection of fluoroquinolone-resistant *Campylobacter coli* in a broiler breeder flock and the commercial broiler chicken progeny of this flock.** We cultured isolates of *C. jejuni* and *C. coli* from a flock of broiler breeder chickens that had been treated with a poultry fluoroquinolone antibiotic 1 week prior to sampling. The *C. jejuni* isolates exhibited ciprofloxacin MICs of $<0.25$ μg/ml; however, the MIC for the *C. coli* isolates was 16 μg/ml. The *C. coli* isolates were typed by RAPD-PCR in order to determine the diversity of strains that were cultured. All of the *C. coli* isolates produced the same RAPD DNA pattern (data not shown).

In order to investigate whether the ciprofloxacin-resistant *C. coli* strain had also colonized broiler chicks originating from the parental flock, we sampled a flock of 6-week-old broiler chickens that were hatched from the treated breeder flock. Four *C. coli* isolates were detected in the commercial broiler flock on plates containing ciprofloxacin. RAPD-PCR typing indicated that the breeder flock and the descendants of this flock were colonized with the same strain of *C. coli* (data not shown), and definitive confirmation was obtained by PFGE using two restriction enzymes (Fig. 1B and C). The *C. coli* isolates from the broiler flock produced the same PFGE fingerprints that the isolates from the breeders produced. In addition, antimicrobial susceptibility testing confirmed that the *C. coli* isolates from the broilers exhibited drug susceptibility

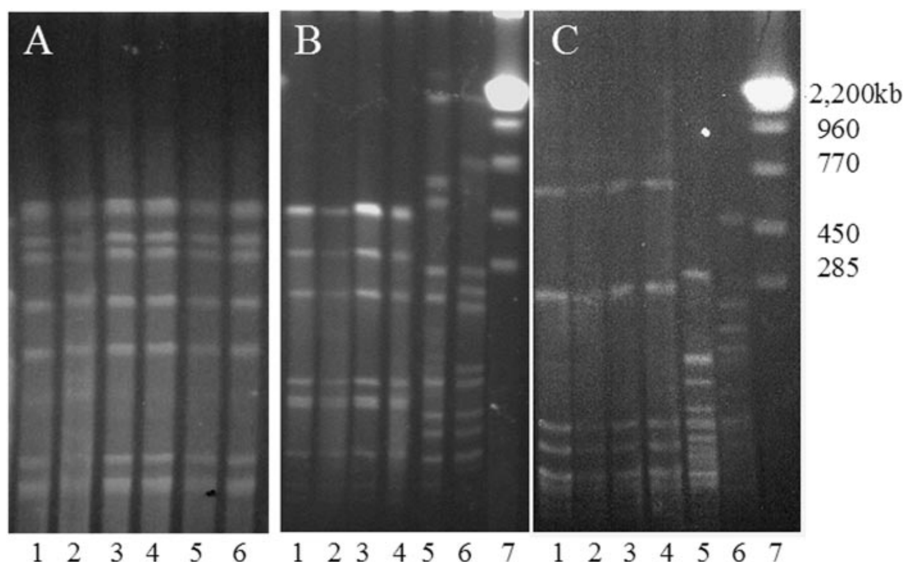


FIG. 1. Strain typing of *C. jejuni* isolates by PFGE. (A) Isolates from two different commercial broiler farms. Lanes 1 to 3 contained isolates from farm CF-3, and lanes 4 to 6 contained isolates from farm CF-1. Genomic DNA was digested with SmaI. (B and C) SmaI (B) and KpnI (C) typing of *C. coli* isolated from a broiler breeder flock and the commercial broiler progeny of this flock. Lanes 1 and 2 contained *C. coli* isolated from breeder parental chickens, lanes 3 and 4 contained *C. coli* isolated from their 6-week-old progeny, lanes 5 and 6 contained *C. jejuni* isolated from the progeny broilers, and lane 7 contained *S. cerevisiae* molecular weight markers.

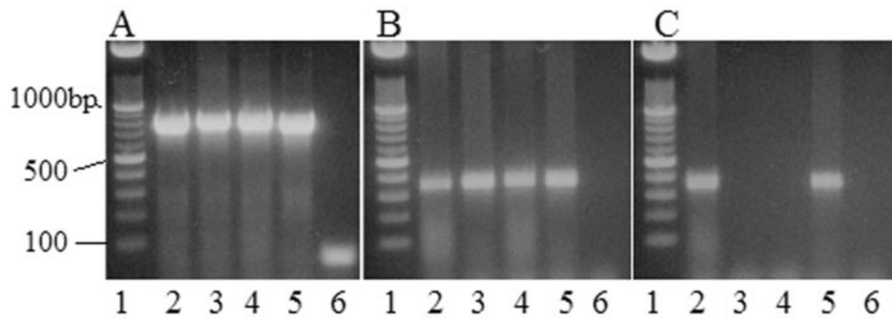


FIG. 2. PCR detection of *Campylobacter* species present in the intestinal and yolk contents of chicks on the day of hatching. Ten embryonating eggs were obtained from a sarafloxacin-treated broiler breeder flock and hatched in a research hatching cabinet. In addition, 10 chicks from this flock were obtained from the commercial hatchery. On the day of hatching, the ileal, cecal, and yolk contents were aseptically removed, and the DNA was extracted. PCR targeting *Campylobacter* was performed with pooled samples, and the amplicons were separated on a 1.5% agarose gel. (A) 16S rRNA PCR results for chicks hatched in the research cabinet. Pooled yolk, ileum, and cecum samples contained *Campylobacter* DNA. Lane 1 contained DNA molecular weight markers, lane 2 contained *C. jejuni* ATCC 33560 as the positive control, lane 3 contained ileal contents, lane 4 contained cecal contents, lane 5 contained yolk contents, and lane 6 contained a control (no DNA template). (B and C) PCR results for chicks hatched in the research facility (B) and for chicks obtained from the commercial hatchery (C), obtained by using primers specific for the *ceuE* gene of *C. coli*. Pooled yolk, ileum, and cecum samples from chicks hatched in the research facility contained *C. coli* DNA, while only the pooled yolk samples from the commercial hatchery were positive. Lane 1, DNA molecular weight markers; lane 2, *C. coli* M1-19 (positive control); lane 3, ileal contents; lane 4, cecal contents; lane 5, yolk contents; lane 6, control containing no DNA template.

patterns identical to those exhibited by isolates from the breeder flock; the ciprofloxacin and sarafloxacin $MIC_{90}$ was 16 μg/ml, the enrofloxacin $MIC_{90}$ was 8 μg/ml, and the tetracycline $MIC_{90}$ was 64 to 128 μg/ml. These findings indicate that the same antibiotic-resistant *C. coli* strain can be found in both parental and progeny flocks.

**Detection of *Campylobacter* in day-of-hatching chicks.** In order to investigate whether the chicks contained *Campylobacter* prior to placement on the farm, we received day-of-hatching chicks that were obtained from eggs laid by the fluoroquinolone-treated breeder flock. In addition, to investigate whether hatchery contamination contributed to *Campylobacter* colonization, we also hatched eggs from this flock in a sanitized research hatching cabinet at our facility. None of these chicks had access to food or water prior to euthanasia and sampling.

We aseptically removed yolk and intestinal contents from the day-of-hatching chicks for culture and molecular detection of *Campylobacter*. No campylobacter-like colonies were detected by culturing any of the samples from these chicks. However, PCR demonstrated the presence of *Campylobacter* DNA in the ileal, cecal, and yolk contents of chicks hatched in the research hatching cabinet (Fig. 2A). *C. coli*-specific PCR indicated the presence of *C. coli* (Fig. 2B), but *C. jejuni* amplicons were not detected (data not shown). Likewise, *C. coli* DNA was also detected in the yolk of chicks obtained from the commercial hatchery (Fig. 2C). In addition, DNA sequencing of the *Campylobacter*-specific 16S rRNA libraries that were produced from amplification of DNA extracted from the chick intestinal and yolk contents revealed sequences that exhibited the highest levels of similarity to *C. coli*, *C. jejuni*, and *C. lari* (Fig. 3). These findings indicate that *Campylobacter* can be present in the intestine of very young chicks, but its presence may not be detected by culturing.

**Detection of *Campylobacter* in the ileal and cecal contents of broiler chickens.** Since we detected *C. coli* 16S rRNA sequences in the ilea of 3-day-old chicks in a previous study (23), we used PCR to detect *Campylobacter* in community DNA isolated from birds at different ages. In order to evaluate the diversity of *Campylobacter* species detected, the amplicons were also cloned, sequenced, and compared to the 16S rRNA sequences acquired from the day-of-hatching chicks (Fig. 3). *Campylobacter* species were detected in the bacterial community of the ileum at all sample times; however, cecal samples were negative until the birds were 21 days old. These results indicate that *Campylobacter* may initially colonize the small intestine of broilers and that the colonization niche may expand with maturation of the birds. The results of our molecular ecology studies concurred with this finding and demonstrated that the cecal bacterial community was derived from the ileal community during the first 14 days after the birds hatched (23). Since many studies that investigate the epidemiology of *Campylobacter* on broiler farms use feces or cecal droppings for culture detection, this finding may explain why commercial flocks are often culture negative before the chickens are 3 weeks old (15, 28, 29, 30, 31).

## DISCUSSION

*C. jejuni* and *C. coli* are significant food-borne pathogens, and consumption of poultry is a significant risk factor for contracting disease (33, 40). *Campylobacter* infection can cause symptoms that persist long enough to require antimicrobial therapy; therefore, resistant strains are a significant public health concern (38, 42). Figure 4 shows the potential sources of antibiotic-resistant *Campylobacter* for poultry. Many studies have shown that chickens become colonized with *Campylobacter* present in their environment (15, 21, 28, 31), but egg transmission has been hypothesized because some genetic types that colonize broilers cannot be identified in the house environment prior to arrival of the broiler chicks (28, 30, 31). Identical *Campylobacter* strains have been detected in breeder and broiler flocks (9), although the results of the vast majority of studies have not supported the hypothesis that vertical transmission makes a significant contribution to the dissemi-

FIG. 3. Phylogenetic tree showing the relatedness of chick intestinal 16S rRNA sequences to sequences of *Campylobacter* species. The 16S rRNA clone libraries were produced by amplification of DNA extracted from the ileum, cecum, and yolk contents of day-of-hatching chicks and from the ileal and cecal contents of chickens that were 3 to 49 days old (23). The tree was constructed by neighbor-joining analysis of a distance matrix obtained from a multiple-sequence alignment of clone library sequences and GenBank ε-proteobacterial 16S rRNA sequences. Bootstrap values (expressed as percentages of 100 replications) are indicated at branch points. Branches labeled with DOH represent sequences obtained from the clone library from day-of-hatching chicks; branches labeled ileum or cecum (but without DOH) represent sequences obtained from clone libraries of older chickens. Branches labeled with a specific organism's name and accession number represent sequences obtained from the GenBank database.

nation of *C. jejuni* on poultry farms (15, 19, 20, 21, 28, 29, 31, 32). In this study, we were able to detect *C. coli* DNA in the ileum, cecum, and yolk contents of chicks on the day of hatching. Since these birds had not received food or water prior to sampling, the bacteria were probably present within or on the surface of the egg at the time of hatching. Hatching eggs vary in the level of surface fecal contamination, and several studies have demonstrated that *C. jejuni* can be detected in the inner shell and membranes of eggs, revealing a possible route of exposure for hatching chicks (1, 11, 35). Furthermore, multiple studies have shown that *C. jejuni* can colonize the oviduct of hens (3, 4, 7) and can be cultured from the semen of roosters and toms (7, 10). It is known that a related species of *Campylobacter*, *C. fetus*, can be venereally transferred and cause reproductive infections in livestock (34). The infection correlates with expression of an antigenically variable group of proteins that form an S-layer on the surface of the bacterial cell (8, 16). The production of S-layers has not been described for *C. jejuni* or *C. coli*, and the published genome sequences of two human isolates of *C. jejuni* do not contain *sag* loci that code for an

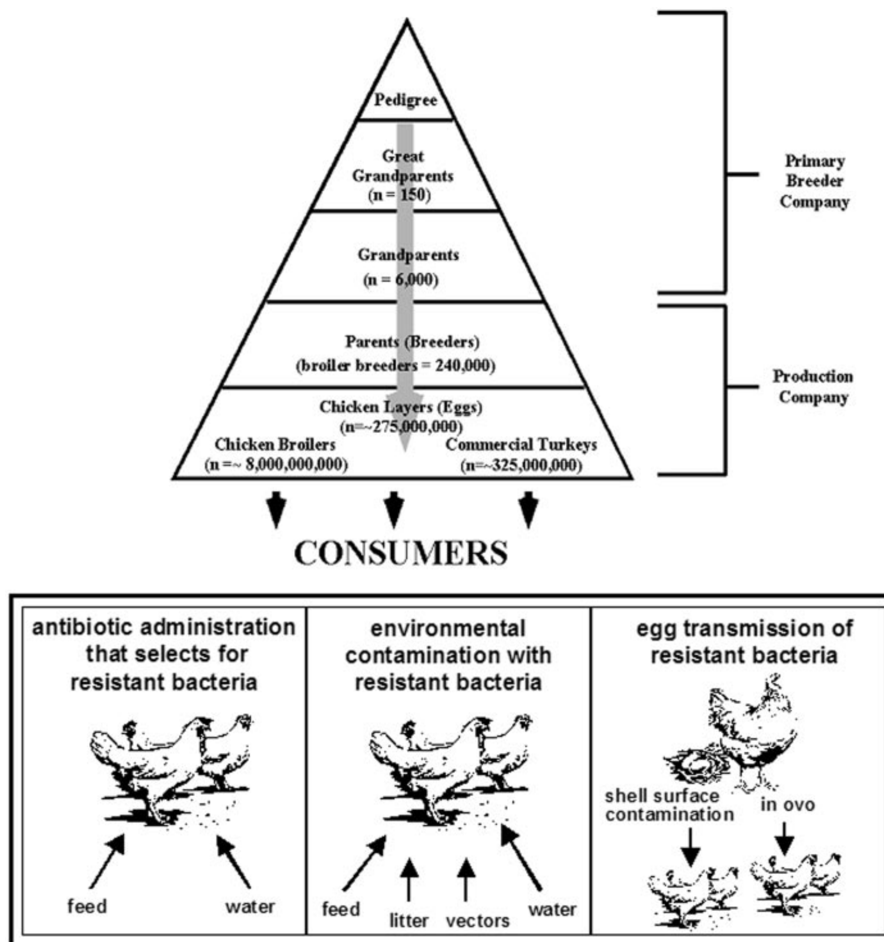


FIG. 4. (Top panel) Integrated structure of the commercial poultry production system. Production companies acquire breeder flocks from the pedigree flocks owned by three to five companies worldwide. These breeder flocks produce hatching eggs that become meat birds and layers for human consumption. (Lower panel) Potential sources of antibiotic-resistant bacteria for poultry. While antibiotic use may select for resistant populations (left box), young chicks lacking a stable intestinal bacterial community may be more susceptible to colonization with resistant strains present in the hatchery or on the farm (middle box). Exposure of hatchlings to resistant bacteria present on the surface of the egg can potentially result in dissemination of resistant strains from the breeder flock to progeny (right box).

S-layer. However, it is not clear whether the distribution of *sag* genes has been investigated for animal isolates of either *C. jejuni* or *C. coli*.

In our study, a common source of *Campylobacter* was revealed by culture detection of a ciprofloxacin-resistant *C. coli* strain that was isolated from both progeny broilers and the parent flock. This strain could have been acquired by horizontal transmission from a common environmental source. However, Pearson et al. (31) postulated that there was low-level vertical transmission of *C. jejuni* based on detection of the same serotypes in the hatchery and in the broilers examined. These strains could be masked by the more prevalent strains already present in the broiler house environment. It was through a fortuitous opportunity that we had a selectable marker, ciprofloxacin resistance, that may have enhanced our ability to isolate the *C. coli* described in this study. Most epidemiology studies utilize culturing as the primary mechanism of detection of *C. jejuni*. However, many selective agar media used for isolation may not consistently detect all *Campylobacter* isolates (37). In our study, we used several enrichment techniques, including selective filtration rather than selective agar, to isolate *Campylobacter* from young chicks. This approach was not successful for detecting viable *Campylobacter* in the intestine or yolk of day-of-hatching chicks. Our inability to isolate *Campylobacter* colonies might have been due to low numbers present in the chicks, although the medium and incubation conditions may not have been appropriate for culturing from these environments. *Campylobacter* can exist in a viable but nonculturable form in water (41), which can be detected by molecular methods (24). Stern et al. (39) demonstrated that viable but nonculturable cells colonize chickens. Molecular detection in chicks, supported by culture detection later in the life of the flock, allowed us to demonstrate that *C. coli* could be present in the chicks at placement. Furthermore, these findings indicate that integrator sources must be considered when workers evaluate management practices in order to reduce *Campylobacter* colonization of broiler chickens and, specifically, that antibiotic usage in the upper levels of the poultry production pyramid might affect the prevalence and dissemination of drug-resistant *Campylobacter* among meat

birds. It may be necessary to examine poultry production at all levels to better understand the impact of antibiotic use on the emergence of drug-resistant pathogens like *Salmonella* and *Campylobacter*. Further investigations to identify critical control points for limiting the spread of drug-resistant food-borne pathogens in this important food source are warranted.

## ACKNOWLEDGMENTS

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