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Characterization of Fluoroquinolone Resistance among Veterinary Isolates of Avian *Escherichia coli*

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Fluoroquinolone-resistant avian *Escherichia coli* isolates from northern Georgia were investigated for *gyrA* and *parC* mutations. All isolates contained a mutation in GyrA replacing Ser83 with Leu; seven isolates also contained mutations replacing Asp87 with either Gly or Tyr. Random amplified polymorphic DNA analysis revealed that quinolone-resistant *E. coli* isolates were genetically diverse.

Colibacillosis continues to significantly contribute to increased mortality and economic losses in the poultry industry (1, 4, 6, 11). Sarafloxacin and enrofloxacin were approved in 1995 and 1996 in the United States for veterinary use to help control morbidity and mortality associated with *Escherichia coli*-related colibacillosis infections (14).

Quinolone resistance mechanisms employed by gram-negative bacteria include chromosomal mutations that reduce membrane permeability and decrease drug accumulation or alter DNA topoisomerases (9, 12, 17, 22, 23, 25). Clinical resistance to fluoroquinolones in *E. coli*, however, is mostly associated with mutations that result in amino acid changes in the A subunit (*gyrA*) and the B subunit (*gyrB*) of the DNA gyrase and in the *parC*-encoded subunit of topoisomerase IV (5, 8, 17, 18, 20, 23, 25).

This study was undertaken to investigate the genetic mechanisms involved in the emergence of bacterial fluoroquinolone resistance among pathogenic avian *E. coli* isolates. Furthermore, isolates were genetically characterized via random amplified polymorphic DNA (RAPD) analysis to determine if fluoroquinolone resistance was associated with specific *E. coli* clones. Fluoroquinolone resistance was surveyed among avian *E. coli* organisms isolated at the Poultry Diagnostic and Research Center (PDRC), University of Georgia, during a 37-month period from May 1996 to June 1999 (Fig. 1). Five hundred thirty-five *E. coli* isolates from clinical cases of avian colibacillosis were identified at the PDRC during this time. The percentage of sarafloxacin-resistant avian *E. coli* isolates steadily increased from 15% in 1996 to 40% in 1999 (Fig. 1). Dual resistance to sarafloxacin and enrofloxacin increased from 9% in 1997 to 30% in 1999.

Antimicrobial susceptibilities of 29 nalidixic acid-resistant avian *E. coli* isolates were determined with agar dilution and broth microdilution methods and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (15, 16). The majority of *E. coli* isolates were also resistant to several other antimicrobials tested, particularly sulfamethoxazole ($n = 27$), tetracycline ($n = 25$), streptomycin ($n = 24$), gentamicin ($n = 18$), and ampicillin ($n = 16$). Resistance to the cephalosporins cephalothin ($n = 6$) and ceftiofur ($n = 4$) was observed as well. Additionally, nine *E. coli* isolates were resistant to chloramphenicol. Sixty-six percent (19 of 29) of quinolone-resistant *E. coli* isolates exhibited multiple resistance to five or more antimicrobials. MICs of nalidixic acid, enrofloxacin, sarafloxacin, and ciprofloxacin were then determined using an agar plate dilution method (Table 1). The MIC of the antibiotic was defined as the concentration (in micrograms per milliliter of agar) at which no more than two colonies were detected. Ninety-three percent (27 of 29) of the isolates required >256 μg of nalidixic acid/ml for inhibition, whereas two isolates required 64 μg/ml (resistant MIC breakpoint, ≥32 μg/ml). Three isolates required 32 μg of enrofloxacin/ml (resistant MIC breakpoint, ≥2 μg/ml) and 32 μg of sarafloxacin/ml (resistant MIC breakpoint, ≥0.25 μg/ml) for inhibition, and 13 isolates required 8 μg of sarafloxacin/ml or more for inhibition. Six isolates displayed intermediate susceptibility to ciprofloxacin (MIC = 2 μg/ml), and one isolate was cross-resistant to ciprofloxacin (MIC = 16 μg/ml; resistant MIC breakpoint, ≥4 μg/ml) as well as to enrofloxacin (≥32 μg/ml) and sarafloxacin (≥32 μg/ml).

Single-stranded conformational polymorphism PCR was employed to investigate the presence of mutations in the quinolone resistance-determining regions (QRDR) of the *gyrA* gene of DNA gyrase and the *parC* gene of topoisomerase IV as previously described (8). DNA sequencing of the *gyrA* and *parC* regions using previously described primers (8) confirmed the initial single-stranded conformation polymorphism analysis and interpretations (Table 1). All 29 nalidixic acid-resistant *E. coli* isolates contained the amino acid substitution Ser83 → Leu in the GyrA QRDR (Table 1). However, many of these *E. coli* isolates displayed variable fluoroquinolone susceptibility patterns. Seven isolates had the additional amino acid substitution Asp87 → Tyr ($n = 2$) or Asp87 → Gly ($n = 5$) within the GyrA QRDR. Twenty-three of 29 quinolone-resistant *E. coli* isolates assayed contained a silent mutation at Ser85 (AGC→AGT) in *parC*. However, no mutations conferring amino acid substitutions were detected in the QRDR of *parC* among the quinolone-resistant avian *E. coli* isolates (Table 1).

Fluoroquinolone-resistant avian *E. coli* isolates have also been previously identified in Saudi Arabia and Spain (3, 4). However, neither study identified the specific mutations associated with the fluoroquinolone resistance phenotypes. Additionally, Everett et al. observed that the majority of veterinary

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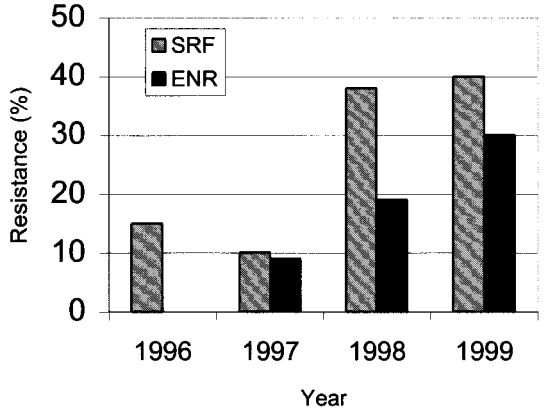

FIG. 1. Emergence of fluoroquinolone resistance among clinical *E. coli* isolates in northern Georgia. Five-hundred thirty-five pathogenic avian *E. coli* isolates implicated in colibacillosis were submitted to the PDRC diagnostic laboratory during a 37-month period from May 1996 to June 1999. Forty-one isolates were submitted from May to December 1996, 189 isolates in 1997, 228 isolates in 1998, and 77 isolates from January to June 1999. Sarafloxacin and enrofloxacin susceptibilities were determined according to NCCLS standards (16). SRF, sarafloxacin; ENR, enrofloxacin.

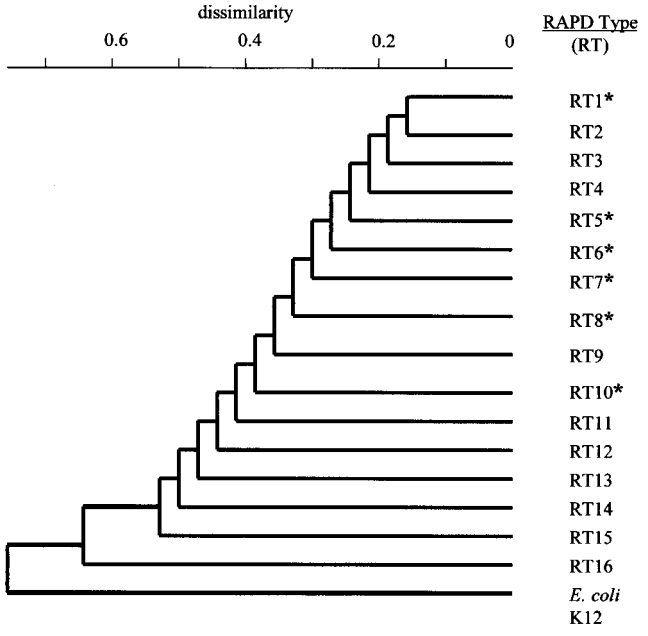

FIG. 2. Fluoroquinolone resistance occurs in genetically diverse avian *E. coli* isolates. Similarities among *E. coli* RAPD patterns were identified by cluster analysis using the neighbor-joining method to draw a phylogenetic tree (13). *E. coli* isolates with similar RAPD patterns were placed into the same cluster or branch and designated as an RT. Phylogenetic analysis identified 16 RTs based on differences in RAPD patterns among 184 avian *E. coli* isolates that have been previously described (13). An asterisk indicates an RAPD type identified among fluoroquinolone-resistant avian *E. coli* isolates.

E. coli isolates (six of eight) resistant to fluoroquinolones isolated in the United Kingdom had mutations only in the QRDR of the *gyrA* gene (8). The present study suggests that avian *E. coli* isolates recovered from diseased poultry in the United States display similar resistance phenotypes as well as sharing common resistance mechanisms with those isolates previously described by other European investigators. However, we cannot exclude additional mechanisms that have yet to be identified which may contribute to fluoroquinolone resistance, especially in isolates with high-level resistance.

Avian *E. coli* isolates were further typed by RAPD using previously published primers (13). Grouping of *E. coli* isolates into each cluster or branch in the dendrogram correlated with similarities in their RAPD DNA pattern (Fig. 2). A total of 16 different clusters or RAPD types (RT) were identified from 184 avian *E. coli* isolates that have been previously described (13). The 29 fluoroquinolone-resistant avian *E. coli* isolates were typed by RAPD and could be assigned to six different RT groups: RT1, RT5, RT6, RT7, RT8, and RT10 (Fig. 2). The random association of fluoroquinolone-resistant avian *E. coli* isolates among multiple RTs indicates a genetically diverse population and suggests that fluoroquinolone resistance has appeared among independent chromosomal backgrounds and is not due to the emergence of particular resistant clonal genotypes.

This study reports the first occurrence of fluoroquinolone resistance in veterinary *E. coli* isolates recovered from clinical cases of avian colibacillosis in the United States. A marked increase in sarafloxacin and enrofloxacin resistance was observed among pathogenic avian *E. coli* in northern Georgia from 1996 to 1999. This trend coincides with the approval of these fluoroquinolones in 1995 and 1996 for treatment of *E. coli*-related poultry infections. Similar results have been seen among other pathogenic *E. coli* strains and most likely reflect the selection of antibiotic-resistant populations due to therapeutic use of antimicrobials (3, 4, 6, 7, 20, 24). There is mounting evidence that antimicrobial use in veterinary medicine may select for antimicrobial-resistant zoonotic bacterial pathogens (e.g., *Salmonella* and *Campylobacter*) (2, 7, 10, 19, 21). This has led to increased pressure to limit fluoroquinolones in animals to preserve the value of these drugs in the treatment of human infections (2, 10, 19, 21). However, the proposed linkage between fluoroquinolone use in agriculture and the occurrence of resistant human enteric bacterial pathogens is still being de-

TABLE 1. GyrA and ParC mutations and fluoroquinolone susceptibility profiles of avian *E. coli* isolates

No. of isolates	Mutation in:		MIC range (μg/ml)[a]			
	GyrA protein[b]	ParC gene[c]	Nal	Cip	Enr	Sar
18	Ser83 → Leu	AGC → AGT	64–>256	0.5 to 16	1–32	1–32
4	Ser83 → Leu	None (wild type)	>256	1	2	4
3	Ser83 → Leu; Asp87 → Gly	AGC → AGT	>256	1	2	4–8
2	Ser83 → Leu; Asp87 → Gly	None (wild type)	>256	1	2	2–4
2	Ser83 → Leu; Asp87 → Tyr	AGC → AGT	>256	1	2	4–8

[a] MICs determined with agar plate dilution methods according to NCCLS guidelines (15, 16). Nal, nalidixic acid; Cip, ciprofloxacin; Enr, enrofloxacin; Sar, sarafloxacin.
[b] Amino acid substitution(s) in GyrA.
[c] Nucleotide substitution in the third position of the Ser58 codon.

bated (19). Regardless, the detection of fluoroquinolone bacterial resistance in a veterinary situation stresses the need for the judicious use of these antimicrobials.

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