

Comparison of the Prevalences and Antimicrobial Resistances of *Escherichia coli* Isolates from Different Retail Meats in the United States, 2002 to 2008

S. Zhao, K. Blickenstaff, S. Bodeis-Jones, S. A. Gaines, E. Tong, and P. F. McDermott

Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U. S. Food & Drug Administration, Laurel, Maryland, USA

Escherichia coli isolates were recovered from the National Antimicrobial Resistance Monitoring System retail meat program and examined for antimicrobial susceptibility. Retail meat samples ($n = 11,921$) from four U.S. states collected during 2002 to 2008, consisting of 2,988 chicken breast, 2,942 ground turkey, 2,991 ground beef, and 3,000 pork chop samples, were analyzed. A total of 8,286 *E. coli* isolates were recovered. The greatest numbers of samples contaminated with the organism were chicken (83.5%) and turkey (82.0%), followed by beef (68.9%) and pork (44.0%). Resistance was most common to tetracycline (50.3%), followed by streptomycin (34.6%), sulfamethoxazole-sulfisoxazole (31.6%), ampicillin (22.5%), gentamicin (18.6%), kanamycin (8.4%), amoxicillin-clavulanic acid (6.4%), and ceftiofuran (5.2%). Less than 5% of the isolates had resistance to trimethoprim, ceftriaxone, ceftiofur, nalidixic acid, chloramphenicol, and ciprofloxacin. All isolates were susceptible to amikacin. Compared to beef and pork isolates, the poultry meat isolates had a greater percentage of resistance to all tested drugs, with the exception of chloramphenicol, to which pork isolates had the most resistance. More than half of the turkey isolates (56%) were resistant to multi-drugs (≥ 3 classes) compared to 38.9% of chicken, 17.3% of pork, and 9.3% of beef isolates. The *bla*_{CMY} gene was present in all ceftriaxone- and ceftiofur-resistant isolates. The *cmlA*, *flo*, and *catI* genes were present in 45%, 43%, and 40% of chloramphenicol-resistant isolates, respectively. Most nalidixic acid-resistant isolates (98.5%) had a *gyrA* mutation in S83 or D87 or both, whereas only 6.7% had a *parC* mutation in either S80 or E84. The results showed that *E. coli* was commonly present in the retail meats, and antimicrobial resistance profiles differed according to the animal origin of the isolates.

Escherichia coli is a commensal bacterium in humans and animals and has a wide range of hosts. It is commonly present in the environment and is considered an indicator of fecal contamination in food and water. *E. coli* can acquire, maintain, and transmit resistance genes from other organisms in the environment. Due to its ubiquity in humans and animals and its role as a pathogenic and commensal organism, *E. coli* has become one of the microorganisms that are commonly resistant to antimicrobials.

The level of antimicrobial resistance in *E. coli* represents a useful indicator of resistance dissemination in bacterial populations and of selective pressure imposed by the antimicrobials used in treatment of food animals and humans (2, 25, 28, 30). Sáenz et al. (25) showed that the frequency of resistance to different antimicrobials in *E. coli* differed according to the source of the isolates. *E. coli* isolates from broilers were resistant to ciprofloxacin (CIP) (38%) and gentamicin (GEN) (40%) compared to 0% resistance to both drugs among *E. coli* isolates from healthy human volunteers versus 16% and 8% resistance among *E. coli* isolates from human clinical specimens. Kikui et al. (22) reported that *E. coli* isolates from chicken had higher resistance than *E. coli* isolates from pigs and cattle.

The emergence of antimicrobial resistance among *E. coli* strains of animal origin has important public health implications. Several studies showed that drug-resistant *E. coli* infections in humans were caused by strains from animals and that those infectious agents harbored the same mobile resistance genes as were found in diverse bacterial species from a variety of animal sources (17, 19, 20, 35). Johnson et al. investigated antimicrobial-resistant *E. coli* isolates from humans and poultry products in Minnesota and Wisconsin (20) and demonstrated that phylogenetic and virulence markers of antimicrobial-susceptible human isolates dif-

fered considerably from those of antimicrobial-resistant isolates from humans and poultry. However, antimicrobial-resistant isolates from humans were similar to those from poultry whereas antimicrobial-susceptible and antimicrobial-resistant isolates from poultry were largely indistinguishable. Those researchers concluded that some antimicrobial-resistant *E. coli* isolates from humans may have originated from poultry, whereas antimicrobial-resistant *E. coli* isolates from poultry likely were derived from susceptible *E. coli* strains in poultry. A similar study conducted by Vieira et al. (34) showed that resistance in *E. coli* isolates from poultry and pigs was highly correlated with resistance in *E. coli* isolates from human bloodstream samples. The study supported the hypothesis that a large proportion of resistant *E. coli* isolates causing bloodstream infections in humans may be derived from food sources.

Due to its natural presence in the gut of animals and easy acquisition of antimicrobial resistance, *E. coli* has been selected as a sentinel organism in antimicrobial resistance monitoring programs worldwide. In the United States, the Food & Drug Administration (FDA) National Antimicrobial Resistance Monitoring System (NARMS) tracks antimicrobial resistance in food-borne pathogenic and commensal bacteria from different sources along the food supply chain. The objectives of this study were to deter-

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Address correspondence to S. Zhao, shaohua.zhao@fda.hhs.gov.

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mine the prevalence, antimicrobial susceptibility, and mechanisms of selected resistance phenotypes of *E. coli* isolates from raw retail meats purchased in the United States during 2002 to 2008.

MATERIALS AND METHODS

Retail meat sampling. Meat samples were purchased at retail outlets and cultured for *E. coli* at participating public health laboratories in Georgia (GA), Maryland (MD), Oregon (OR), and Tennessee (TN). Each site obtained up to 40 retail meats per month from grocery stores as follows: 10 samples each of chicken breast (CB) with skin on, ground turkey (GT), ground beef (GB), and pork chops (PC). A convenience sampling approach was used from 2002 to 2004 in which samples were collected from grocery stores in close proximity to the testing sites. A stratified random sampling scheme has been used since 2005 whereby samples were collected from a geographically coded list of grocery stores within ZIP codes representing highly populated areas for each site. The ZIP codes were partitioned into quadrants, and grocery stores were randomly selected for sample collection using SAS version 9.1.3 software. For each sample, the test sites logged the store name, lot number (if available), sell-by date, purchase date, and laboratory processing date.

Microbiological analysis. In each laboratory, samples were refrigerated at 4°C and were processed no later than 96 h after purchase. Retail meat and poultry packages were kept intact until they were aseptically opened in the laboratory at the start of examination. For chicken and pork cuts, one piece of meat was examined. For ground beef and turkey products, 25-g portions were used. Each sample was placed in a sterile plastic bag with 225 ml of buffered peptone water, and the bags were vigorously shaken. Rinsate (50 ml) from each sample was transferred to a sterile flask for isolation and identification of *E. coli*. Double-strength MacConkey broth (50 ml) was then added, and the contents were mixed thoroughly and incubated at 35°C for 24 h.

From each flask, one loop of overnight broth culture was streaked onto a Levine's eosin methylene blue (EMB) agar plate and incubated at 35°C for 24 h. When typical *E. coli* colonies were present, one well-isolated colony was selected and streaked onto a Trypticase soy agar plate supplemented with 5% defibrinated sheep blood (Remel, Lenex, KS). After indole and oxidase tests, *E. coli* isolates were subsequently frozen at -60 to -80°C in Brucella broth with 20% glycerol and shipped in cryovials on dry ice to the laboratory of the FDA Administration Center for Veterinary Medicine (FDA-CVM). Upon arrival, every isolate was streaked for purity on a blood agar plate (BAP) before being confirmed as *E. coli* by the use of a Vitek microbial identification system (bioMérieux, Hazelwood, MO). Bacteria were stored in Trypticase soy broth containing 15% glycerol at -80°C until use. All bacterial media were obtained from Becton Dickinson and Company, Franklin Lakes, NJ, unless otherwise specified.

Antimicrobial susceptibility testing. Antimicrobial MICs were determined using a Sensititre automated antimicrobial susceptibility system in accordance with the instructions of the manufacturer (Trek Diagnostic Systems, Cleveland, OH). Initially, all isolates were tested using a panel designed for NARMS, which included ceftriaxone (AXO), ceftiofur (TIO), amoxicillin-clavulanic acid (AMC), ampicillin (AMP), cefoxitin (FOX), ciprofloxacin (CIP), nalidixic acid (NAL), amikacin (AMI), gentamicin (GEN), streptomycin (STR), kanamycin (KAN), sulfisoxazole (FIS), trimethoprim-sulfamethoxazole (COT), doxycycline (DOX), tetracycline (TET), and chloramphenicol (CHL). A total of 273 TIO^r strains were tested with a secondary panel of β -lactam antimicrobials that included aztreonam (AZT), ceftinome (CQN), imipenem (IMI), cefepime (FEP), piperacillin-tazobactam (P/T), ceftazidime (TAZ), ceftazidime-clavulanic acid (T/C), cefotaxime (FOT), and cefotaxime-clavulanic acid (F/C). Results were interpreted in accordance with CLSI criteria (8), with the exception of streptomycin (resistance breakpoint ≥ 64 $\mu\text{g/ml}$) and ceftinome (resistance breakpoint ≥ 32 $\mu\text{g/ml}$). *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas*

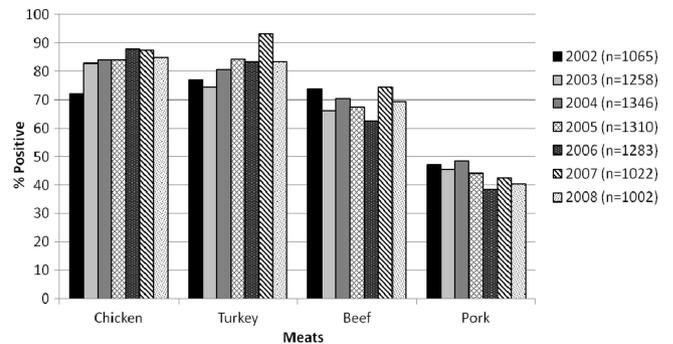


FIG 1 Prevalence of *E. coli* recovered from retail meats in 2002 to 2008. A total of 11,921 retail meat samples, consisting of 2,988 chicken breast, 2,942 ground turkey, 2,991 ground beef, and 3,000 pork chop samples, were analyzed during 2002 to 2008. A total of 8,286 *E. coli* isolates (2,494 from chicken breast, 2,412 from ground turkey, 2,061 from ground beef, and 1,319 from pork chops) were recovered.

aeruginosa ATCC 27853 were used as quality control organisms in the antimicrobial MIC determinations.

DNA isolation, PCR, and DNA sequencing. A total of 273 TIO^r isolates were screened for the presence of *bla*_{CMY}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, and *bla*_{CTX-M} and 172 CHL^r isolates were screened for the presence of *cmlA*, *flo*, *catI*, *catII*, and *catIII* genes by PCR using previously published methods (4, 6, 21, 26, 33, 43). A total of 269 Nal^r *E. coli* isolates were screened for the presence of *qnrA*, *qnrB*, *qnrS*, and *acc(6')-Ib-cr* genes and analyzed for quinolone resistance-determining region (QRDR) point mutations in *gyrA* and *parC*. The primers and PCR conditions were as previously reported (11, 14, 24). DNA templates were prepared using MoBio Ultraclean DNA isolation kits (MoBio Laboratories, Inc., Carlsbad, CA) following the manufacturer's instructions. Amplifications were carried out using 200 ng of DNA template, 250 μM (each) deoxynucleoside triphosphates, 1.5 mM MgCl₂, 50 pmol of each primer, and 1 U of AmpliTaq Gold Taq polymerase (Applied Biosystems, Foster City, CA). The amplified products were separated by gel electrophoresis on 1.0% agarose gels and stained with ethidium bromide. Each PCR product was sequenced using an ABI 3730 automated sequencer (Applied Biosystems). Sequences were analyzed using the BLAST network service of the National Center for Biotechnology Information. Each gene was identified by comparison to sequences in the GenBank database (accession numbers AF252855, AY816215, EU418734, DQ415372, DQ319067, AY737250, EU979560, J02967, Y16784, X92506, EU279427, EU093091, EU391634, and GQ438248).

Statistical analysis. Statistical analyses were conducted using SAS version 9.2 software (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) with Tukey's multiple-comparison test was applied to evaluate statistically significant differences in the prevalences and antimicrobial resistances of *E. coli* isolates recovered from each meat type. *P* values of <0.05 were considered statistically significant. The Cochran-Armitage trend test was used for trend analysis of prevalence and antimicrobial resistance over time.

RESULTS

Prevalence of *E. coli* in retail meats. From 2002 to 2008, a total of 11,921 meat samples, collected in GA, MD, OR, and TN, were examined for the presence of *E. coli*. The sample set consisted of 2,988 chicken breast, 2,942 ground turkey, 2,991 ground beef, and 3,000 pork chop samples. Overall, 69.5% ($n = 8,286$) of the 11,921 retail meat samples were positive for *E. coli*, with the highest prevalence in chicken breast samples ($n = 2,494$; 83.5%), followed by ground turkey ($n = 2,412$; 82.0%), ground beef ($n = 2,061$; 68.9%), and pork chops ($n = 1,319$; 44%) (Fig. 1). There were

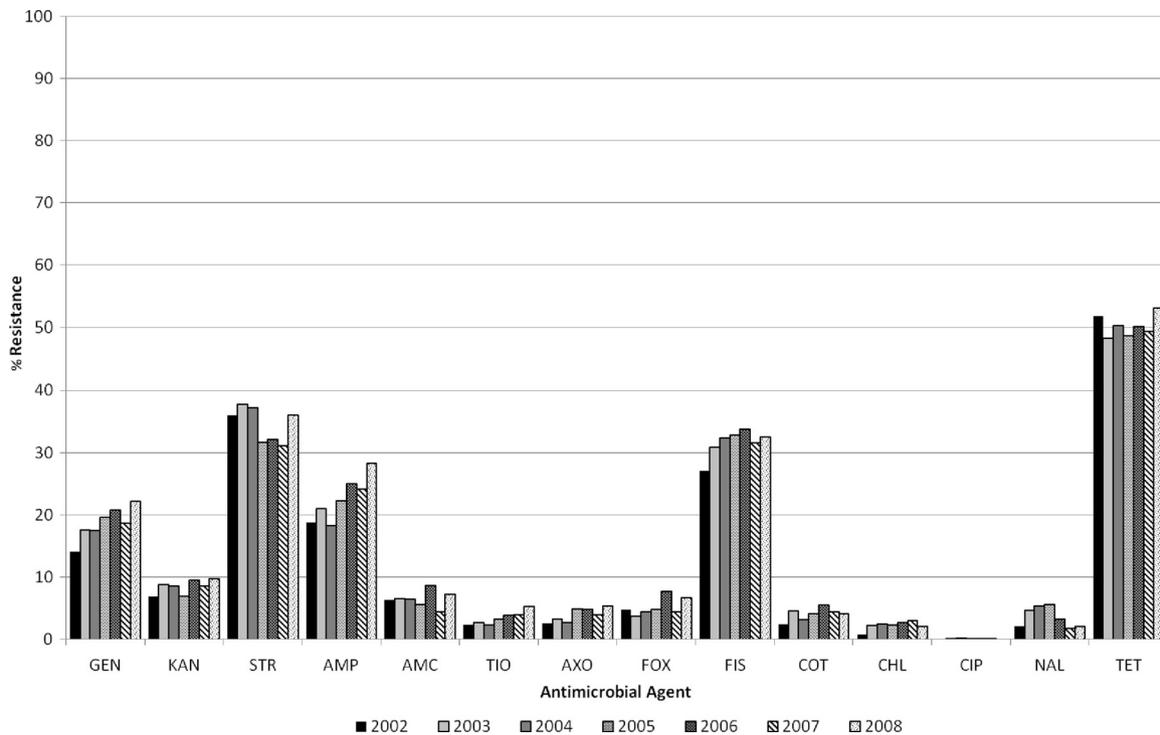


FIG 2 Antimicrobial resistance trends of *E. coli* isolated from retail meats in 2002 to 2008. A total of 8,286 *E. coli* isolates, including 1,065 in 2002, 1,258 in 2003, 1,346 in 2004, 1,310 in 2005, 1,283 in 2006, 1,022 in 2007, and 1,002 in 2008, were tested. The testing was done by broth microdilution using amoxicillin-clavulanic acid (AMC), amikacin (AMI), ampicillin (AMP), ceftiofur (FOX), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole-sulfisoxazole (SMX/FIS), tetracycline (TET), trimethoprim-sulfamethoxazole (COT), and ceftiofur (TIO).

significantly higher isolation rates from poultry meats than from beef and pork ($P < 0.05$). No significant differences in *E. coli* isolation rates were found by month within a sampling site over the 7 years of the study, although some months had slightly higher isolation rates than others (data not shown). However, *E. coli* isolation rates were significantly different among the states ($P < 0.05$). Over the testing time period, GA maintained the highest positive isolation rate (mean of 81.1%), followed by MD (72.8%), TN (68.1%), and OR (53.1%).

Antimicrobial susceptibility. From 2002 to 2008, the *E. coli* isolates from retail meats displayed resistance most frequently to DOX/TET (48.3% to 53.0%), followed by STR (31.1% to 37.2%), FIS (27.1% to 33.8%), AMP (18.3% to 28.3%), GEN (14.1% to 22.2%), KAN (6.9% to 9.7%), AMC (4.4% to 8.7%), FOX (3.7% to 7.7%), COT (2.4% to 5.5%), AXO (2.5% to 5.4%), NAL (1.7% to 5.6%), TIO (2.3% to 5.3%), CHL (0.8% to 3.0%), and CIP (0.0% to 0.2%) (Fig. 2). All isolates were susceptible to AMI.

Resistance profiles of the *E. coli* isolates differed significantly by the type of meat from which they were isolated. The poultry meat isolates had greater resistance to most tested drugs compared to pork and beef isolates (Fig. 3 and Table 1). Notably, the resistance of CB isolates was significantly greater to AMC (11.2%; $P < 0.0001$), FOX (10%; $P < 0.0001$), AXO (8.6%; $P < 0.0001$), and TIO (7.8%; $P < 0.0001$), and the resistance of GT isolates was significantly greater to KAN (15.3%; $P < 0.0001$), AMP (40.9%; $P < 0.0001$), FIS (49.2%; $P < 0.0001$), TET (78.5%; $P < 0.0001$), and CIP (0.3%; $P = 0.0047$). PC isolates had higher resistance to CHL (3.9%) compared to other meat isolates; the resistance level

was significantly higher than that of CB isolates (1.2%; $P = 0.0068$) but not significantly higher than those of GT (2.5%) and GB (2.1%) isolates. In terms of multidrug resistance (MDR [resistance to ≥ 3 antimicrobial classes]), GT isolates had significantly higher MDR (56%) than CB (38.9%), PC (17.3%), and GB (9.3%) isolates. GT isolates also had the highest percentage of resistance to ≥ 5 antimicrobial classes (7.6%) compared to CB (6.6%), PC (2.6%), and GB (1.6%) isolates. An MDR-AmpC profile (resistance to AMP, CHL, STR, FIS, TET, and TIO plus other β -lactams) was detected in *E. coli* isolates from all four types of meats, with the highest number in CB samples (data not shown). A total of 273 TIO^r isolates were tested for resistance to an additional seven β -lactam antimicrobials and screened (using T/C and F/C) for the presence of extended-spectrum β -lactamases (ESBL). All were susceptible to P/T, FEB, CQN, FOT, and IMI. Low resistance to AZT (0.7%) and TAZ (7.0%) was seen. No ESBL-producing *E. coli* isolates were detected.

Presence of resistance genes. The mechanisms of resistance to expanded-spectrum cephalosporins, CHL, and fluoroquinolone (FQ) were investigated at the genetic level. All of the 273 TIO^r/AXO^r isolates contained *bla*_{CMY}, as expected. In addition, 20% ($n = 55$) contained *bla*_{TEM}. No isolates were found to carry *bla*_{SHV}, *bla*_{OXA}, or *bla*_{CTX-M}. Thirty percent ($n = 82$) of TIO^r/AXO^r isolates were resistant to ≥ 9 antimicrobials, and 12% ($n = 33$) were MDR-AmpC. Some of the MDR-AmpC strains also had resistance to GEN, KAN, COT, and/or NAL. Among 172 CHL^r isolates, 45% ($n = 78$), 43% ($n = 74$), and 40% ($n = 68$) carried *cmlA*, *flo*, and *catI*, respectively, whereas only 4.6% ($n = 8$) of isolates carried

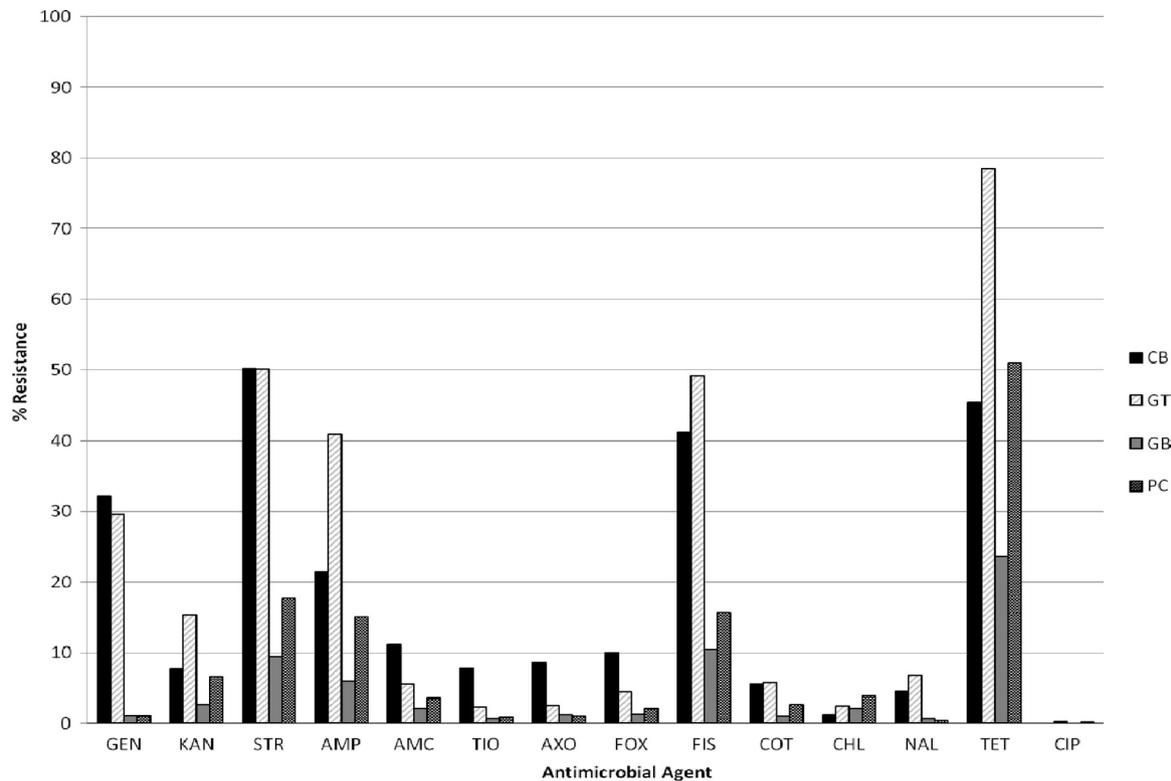


FIG 3 Antimicrobial resistance profiles of *E. coli* recovered from different meat types. A total of 8,286 *E. coli* isolates, including 2,494 from chicken breast (CB), 2,412 from ground turkey (GT), 2,061 from ground beef (GB), and 1,319 from pork chops (PC), were tested. The testing was done by broth microdilution using amoxicillin-clavulanic acid (AMC), amikacin (AMI), ampicillin (AMP), ceftiofur (TIO), cefoxitin (FOX), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole-sulfisoxazole (SMX/FIS), tetracycline (TET), trimethoprim-sulfamethoxazole (COT), and ceftiofur (TIO).

TABLE 1 Antimicrobial resistance of *E. coli* by meat type

Antimicrobial ^a	Breakpoint (μg/ml)	% resistance ^b			
		CB (n = 2,494)	GT (n = 2,412)	GB (n = 2,061)	PC (n = 1,319)
GEN	≥16	32.2 (A)	29.6 (A)	1.1 (B)	1.1 (B)
KAN	≥64	7.7 (A)	15.3 (B)	2.7 (C)	6.6 (A)
STR	≥64	50.2 (A)	50.1 (A)	9.5 (B)	17.7 (C)
AMP	≥32	21.5 (A)	40.9 (B)	6.0 (C)	15 (A)
AMC	≥32/16	11.2 (A)	5.6 (B)	2.2 (C)	3.6 (B, C)
FOX	≥32	10.0 (A)	4.4 (B)	1.3 (C)	2.1 (C)
AXO	≥4	8.6 (A)	2.6 (B)	1.2 (B)	1.0 (B)
TIO	≥8	7.8 (A)	2.4 (B)	0.7 (B)	0.9 (B)
SMX/FIS	≥512	41.2 (A)	49.2 (B)	10.5 (C)	15.7 (D)
COT	≥4/76	5.6 (A)	5.8 (A)	1.0 (B)	2.7 (B)
CHL	≥32	1.2 (A)	2.5 (A, B)	2.1 (A, B)	3.9 (B)
CIP	≥4	0.0 (A)	0.3 (B)	0.0 (A)	0.1 (A)
NAL	≥32	4.5 (A)	6.9 (A)	0.8 (B)	0.4 (B)
TET	≥16	45.4 (A)	78.5 (B)	23.7 (C)	51.0 (D)

^a All isolates were susceptible to amikacin (AMI) (resistance breakpoint > 64 μg/ml), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftiofur (TIO), cefoxitin (FOX), ceftriaxone (AXO), ceftiofur (TIO), SMX/FIS, sulfamethoxazole-sulfisoxazole; COT, trimethoprim-sulfamethoxazole; chloramphenicol (CHL), ciprofloxacin (CIP), nalidixic acid (NAL), and tetracycline (TET). CB, chicken breast; GT, ground turkey; GB, ground beef; PC, pork chops.

^b Entries with identical letters in parentheses indicate no statistically significant differences between those results. Statistical significance calculations are based on $P \leq 0.05$.

catII, *catIII* was not detected in any strains. Seven CHL^r isolates carried three of the five *CHL* genes, and 42 carried two of the five *CHL* genes. Most of the CHL^r isolates were core-resistant to TET (96.5%, 166/172) and FIS (90%, 155/172); 44% ($n = 76$) were ACSSuT (i.e., resistant to AMP, CHL, STR, SMX/FIS, and TET), and 19.2% ($n = 33$) were MDR-AmpC resistant.

A total of 269 NAL^r *E. coli* isolates were screened for the presence of *qnrA*, *qnrB*, *qnrS*, and *acc(6')-Ib-cr* genes and analyzed for QRDR point mutations in the *gyrA* and *parC* genes. Two *qnr*⁺ isolates were found among the NAL^r strains, one carrying *qnrA* and one with *qnrB*. Neither *qnrS* nor *acc(6')-Ib-cr* was detected in any isolates. Additionally, most NAL^r *E. coli* isolates (98.5%) had a *gyrA* mutation(s) in S83 or D87 or both, whereas 6.7% had a *parC* mutation at either S80 or E84 (Table 2). More than half (59.8% and 59.5%) of the NAL^r *E. coli* isolates had core-resistance to FIS and STR, respectively, and 6.3% had core-resistance to TIO and AXO.

DISCUSSION

The use of antimicrobials in food animals, and their role in promoting resistance in food-borne bacteria, is an important public health issue. To measure the baseline resistance rates and the impact of different targeted interventions, an ongoing monitoring system is necessary. As part of the monitoring of commensal and food-borne bacteria, the U.S. NARMS program tracked antimicrobial resistance in *E. coli* isolates from retail meats from four states. We reported on the prevalence, antimicrobial susceptibil-

TABLE 2 Summary of QRDR mutations among 269 NAL^r *E. coli* isolates^a

<i>gyrA</i> mutation	<i>parC</i> mutation	CIP phenotype	NAL phenotype	No. of isolates
S83L	WT	Sensitive	Resistant	218
D87N	WT	Sensitive ^b	Resistant	11
S83L	S80R	Sensitive	Resistant	10
D87Y	WT	Sensitive	Resistant	8
D87G	WT	Sensitive	Resistant	6
S83L/D87N	WT	Resistant	Resistant	4
WT	S80R	Sensitive	Resistant	2
WT	WT	Sensitive	Resistant	1
S83L	E84K	Sensitive	Resistant	1
S83L	S80I	Sensitive	Resistant	1
S83L	S80C	Sensitive	Resistant	1
S83L	S80G	Sensitive	Resistant	1
D87E	WT	Sensitive	Resistant	1
S83A	WT	Sensitive	Resistant	1
S83L/D87N	S80I	Resistant	Resistant	1
S83L/D87G	WT	Sensitive	Resistant	1
WT	E84G	Sensitive	Resistant	1

^a A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; G, glycine; I, isoleucine; K, lysine; L, leucine; N, asparagine; R, arginine; S, serine; Y, tyrosine; WT, wild type.

^b One isolate with this genotype was CIP^r.

ity, and mechanisms of resistance in *E. coli* isolates collected during a 7-year sampling period.

E. coli was commonly present in all four retail meats, signifying a high overall rate of fecal contamination during slaughter and of postslaughter processing contaminations. The highest contamination was in poultry meats (>80%), followed by beef (68.9%) and pork (44%). Other surveys on the prevalence of *E. coli* in retail meats in the United States showed similar findings (18, 39). Johnson et al. (18) reported a survey study of 10 retail markets in the Minneapolis-St. Paul, MN, area during 2001 to 2003 and showed that the contamination rate was 92% in poultry and 69% in beef and pork. Various recovery rates are reported in other studies from the United States and abroad. All have demonstrated that *E. coli* frequently contaminates retail meat products (1, 9, 18, 27, 39).

In general, *E. coli* isolates were more frequently resistant to TET, STR, FIS, AMP, and GEN (19% to 50%) compared with resistance to the other tested agents (<1% to 8.4%). Similar findings of common resistance to TET, FIS, and AMP among *E. coli* isolates from food animals and derived meats were also reported by other investigators (18, 25, 27, 31). The level of resistance to different antimicrobials varied according to the source of the isolates. *E. coli* isolates from poultry meats had a higher resistance rate than those from beef and pork. The resistance of chicken isolates to β -lactams AMC, FOX, AXO, and TIO was significantly greater than that of other meat isolates, whereas the turkey isolates were more resistant to KAN, AMP, FIS, CIP, and TET compared to other meat isolates ($P \leq 0.05$). Similar results were observed with *Salmonella* isolates from retail poultry meat monitoring (40). Although, in general, the pork isolates had lower resistance than the poultry isolates, their resistance to KAN, STR, AMP, FIS, and TET was significantly higher than that of the beef isolates ($P \leq 0.05$). These findings can be considered to reflect, at least in part, the selective pressures imposed by antimicrobial use in different food animal production and processing environments, despite the fact that we do not have antibiotic use histories to correlate with

the susceptibility data. It is speculated that the higher levels of resistance in poultry may be partly due to modern production practices, in which antimicrobials for disease control and prevention are administered through water as well as feed. This imposes greater selection pressure for the development of antimicrobial resistance by exposing large numbers of animals in a treated group (10, 32, 38).

Some notable resistance trends were observed for *E. coli* isolates from certain meat types. Between 2002 and 2008, GT isolates showed a significant increase in resistance to AMP ($P < 0.0001$), TIO ($P < 0.0001$), AXO ($P < 0.0001$), FOX ($P < 0.005$), AMC ($P < 0.0151$), and TET ($P < 0.0089$) and decreased resistance to NAL ($P < 0.0003$). CB isolates showed a significant increase in resistance to GEN ($P < 0.0034$) and decreased resistance to STR ($P < 0.0012$) over the 7-year sampling time frame. Although CB isolates did not show a statistically significant increase in resistance to TIO and AXO, they maintained the highest resistance to these two compounds compared to isolates from other meats, ranging from 5.8% to 11.1% over the years of the study. Both GB and PC isolates were generally stable in resistance levels for all tested antimicrobials (data not shown).

Resistance to expanded-spectrum cephalosporins is a special concern, since these antimicrobials are a front-line therapeutic for treatment of numerous Gram-negative infections, including systemic and pediatric salmonellosis. In the last 10 years, this resistance phenotype has emerged in both humans and food animals in the United States (12, 40, 44). TIO is the only cephalosporin approved for systemic use in food animals in the United States. This drug was first approved in 1988 for treatment of acute bovine respiratory disease and subsequently for use in other food animals, including swine, sheep, chickens, and turkeys (5). It is also used extensively in an off-label manner in broiler chicks as a component of vaccinations. Because TIO^r organisms also exhibit resistance to cephamycins and expanded-spectrum cephalosporins, the use of this antimicrobial in food animals as a selective agent responsible for the emergence and dissemination of AXO^r enteric pathogens such as *Salmonella* has come under increasing scrutiny. The *bla*_{CMY} gene encoding a cephamycinase (an extended-spectrum beta-lactamase) confers resistance to both TIO and AXO (44). Our study showed that all TIO^r/AXO^r isolates carried *bla*_{CMY} but not *bla*_{SHV}, *bla*_{OXA}, or *bla*_{CTX-M}. Similar findings were seen in our previous study on *Salmonella* isolates from NARMS retail meats (40). A major public health concern is that TIO^r/AXO^r isolates are also often MDR. Among the *E. coli* isolates examined here, we found that a very high proportion (30%) of TIO^r/AXO^r isolates were resistant to ≥ 9 antimicrobials and that 12% were MDR-AmpC. The *bla*_{CMY} gene often resides in conjugative MDR plasmids, in some cases being present in multiple copies (36, 40). The poultry meat isolates have shown a steady increase in resistance to TIO and AXO over the years. This has prompted the U.S. Food and Drug Administration to propose limiting the extralabel use of extended-spectrum cephalosporins in order to preserve their effectiveness for treating human infections (13).

A small percentage (1.2% to 3.9%) of *E. coli* isolates from all meat types were resistant to CHL, a drug that was withdrawn from food animal use in the United States in the 1980s due to its toxicity (15, 29). However, CHL resistance has been persistently seen in *E. coli* and *Salmonella* isolates from food animals (3, 37, 40). Florfenicol, a closely related drug, is approved for veterinary use in food animals in the United States. Florfenicol resistance mediated

by the *flo* gene confers nonenzymatic cross-resistance to chloramphenicol (3). This may contribute in part to the persistence of low CHL resistance in the food animal environment. Our data suggest that florfenicol coselection accounts for around 43% of CHL resistance, implying that other selecting factors play a role. In addition, CHL resistance is mediated by *cmlA*, *catI*, *catII*, and *catIII*, and these genes frequently reside on integrons and MDR plasmids (7, 16, 23) in enterics. These mobile elements often contain multiple resistance genes, such as *sul*, conferring resistance to SMX/FIS, and *tet*, conferring TET resistance. Over 90% of CHL^r isolates in the present study had coresistance to TET and SUL/FIS. Therefore, coselection by these drug classes likely also plays an important role in the persistence of CHL resistance. Additionally, 42% and 12% of CHL^r isolates had ACSSuT and MDR-AmpC resistance phenotypes, respectively. These phenotypes have emerged in *Salmonella* in the last 10 to 20 years. Genetic analysis of ACSSuT and MDR-AmpC phenotypes from *Salmonella* showed that *cmlA*, *cat*, and *flo* are often linked with numerous other resistance genes such as *tet*, *sul*, *aadA*, *pse*, *qac*, *sugE*, and *bla*_{CMY}, which are resident on integrons and MDR plasmids (36, 41, 42). The *qac* and *sugE* genes encode resistance to disinfectants (quaternary ammonium compounds), including cetylpyridinium chloride (CPC). These loci are often found on MDR plasmids (36). CPC has been approved for use in chicken slaughterhouses for carcass decontamination. Thus, although on-farm antibiotic use is considered a major cause of antimicrobial resistance in retail meat isolates, consideration must be given to the role of other chemical treatments in slaughterhouses and in retail outlets that can select for strain types carrying CHL and other resistances. Some *E. coli* isolates harbored two or three of five CHL resistance genes, although no additive effect on MIC was found among isolates with multiple alleles (data not shown).

The development of resistance to FQs is also a particular concern, since these antimicrobials are critically important for treating many human infections. Only 6 CIP^r isolates were found among 269 NAL^r isolates, and all 6 were isolated from GT. The NAL^r isolates had decreased susceptibility to CIP, with MICs ranging from 0.125 to 2 µg/ml. All had QRDA mutations in *gyrA* or *parC* or both. Because of rising quinolone resistance in *Campylobacter*, approval of fluoroquinolones for use in poultry was withdrawn from the market in September 2005. Our data show that the proportion of NAL^r *E. coli* strains isolated from chicken breast dropped from 6.6% in 2005 to 2.9% in 2009. Among ground turkey isolates, NAL resistance declined from 10.4% in 2005 to 2.6% in 2009.

In summary, the present report demonstrates a high prevalence of *E. coli* in retail meats, indicating that fecal contamination at slaughter and processing is very common and highlighting the importance of consumer awareness of safe food handling and cooking. The relative levels and types of resistance in *E. coli* (including MDR) from different sources represent a useful indicator of selection pressure imposed by antimicrobials used in different food animals and perhaps processing plants. Since retail meats represent a point of exposure close to the consumer, monitoring the prevalence of antimicrobial resistance among food-borne pathogenic and commensal organisms from such commodities is necessary to identify emerging resistance problems in the food supply. The integration of antimicrobial susceptibility and genetic analysis provides important information on the dissemination, linkage, and recombination of genes underlying MDR in food-

borne bacteria in the animal production environment, retail foods, and humans.

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