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Patterns of Antimicrobial Resistance Observed in *Escherichia coli* Isolates Obtained from Domestic- and Wild-Animal Fecal Samples, Human Septage, and Surface Water

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A repeated cross-sectional study was conducted to determine the patterns of antimicrobial resistance in 1,286 *Escherichia coli* strains isolated from human septage, wildlife, domestic animals, farm environments, and surface water in the Red Cedar watershed in Michigan. Isolation and identification of *E. coli* were done by using enrichment media, selective media, and biochemical tests. Antimicrobial susceptibility testing by the disk diffusion method was conducted for neomycin, gentamicin, streptomycin, chloramphenicol, ofloxacin, trimethoprim-sulfamethoxazole, tetracycline, ampicillin, nalidixic acid, nitrofurantoin, cephalothin, and sulfisoxazole. Resistance to at least one antimicrobial agent was demonstrated in isolates from livestock, companion animals, human septage, wildlife, and surface water. In general, *E. coli* isolates from domestic species showed resistance to the largest number of antimicrobial agents compared to isolates from human septage, wildlife, and surface water. The agents to which resistance was demonstrated most frequently were tetracycline, cephalothin, sulfisoxazole, and streptomycin. There were similarities in the patterns of resistance in fecal samples and farm environment samples by animal, and the levels of cephalothin-resistant isolates were higher in farm environment samples than in fecal samples. Multidrug resistance was seen in a variety of sources, and the highest levels of multidrug-resistant *E. coli* were observed for swine fecal samples. The fact that water sample isolates were resistant only to cephalothin may suggest that the resistance patterns for farm environment samples may be more representative of the risk of contamination of surface waters with antimicrobial agent-resistant bacteria.

Antimicrobial agent resistance has been recognized as an emerging worldwide problem in both human and veterinary medicine, and antimicrobial agent use is considered the most important factor for the emergence, selection, and dissemination of antimicrobial agent-resistant bacteria (29, 51). The principle behind the development of resistance is that bacteria in the guts of humans and animals are subjected to different types, concentrations, and frequencies of antimicrobial agents. Over time, selective pressure selects resistant bacteria that have specific fingerprints for resistance to the antimicrobial agents that have been used (33, 45).

There are four general mechanisms of resistance, all of which are controlled by the action of specific genes: enzymatic inactivation or modification of antimicrobial agents, impermeability of the bacteria cell wall or membrane, active expulsion of the drug by the cell efflux pump, and alteration in target receptors (33). Bacteria gain antimicrobial agent resistance genes through mobile elements, such as plasmids, transposons, and integrons (33, 37), which result in mutations in genes responsible for antimicrobial agent uptake or binding sites (43) or activation of portions of bacterial chromosomes (1, 17). Once acquired, resistance genes can be transferred between bacteria, and the ability of *Escherichia coli* to transfer antimicrobial drug resistance is well known (36).

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Antimicrobial agents are used therapeutically in animals and humans for control of bacterial infections and may be incorporated into commercial livestock and poultry feed at subtherapeutic doses for growth promotion. This practice is believed to enhance selection of resistant bacteria more than the therapeutic use of antimicrobial agents in response to clinical disease (47), and it may contribute to antimicrobial agent resistance in humans acquired through the human food chain (2, 51). One strategy to minimize this problem that has been recommended is to stop the use of agents needed for human treatment as feed additives (36, 44, 52), but there is an ongoing debate concerning whether and to what extent feed additives contribute to the development of resistance in human bacterial pathogens (6, 33). To approach this question, several studies have described antimicrobial agent resistance profiles of *E. coli* strains isolated from foods of animal origin, various species of animals, and humans (4, 13, 14, 22, 23, 25, 39, 47).

In addition to the consequences for human health, concerns have been raised about the contamination of surface water with resistant bacteria from livestock operations and human septage. Resistant bacteria have been isolated from a variety of sources, including domestic sewage, drinking water, rivers, and lakes (20, 24, 26). The levels of antimicrobial agent resistance that have been reported range from 72% (26) up to 100 and 87% for fecal and nonfecal coliforms, respectively (24). One study found that livestock contributed more than humans to fecal coliform contamination of surface water and that reducing livestock access to surface water reduced the fecal coliform levels by an average of 94% (18).

Resistance of a single bacterial isolate to more than one antimicrobial drug is commonly reported. Multiple antimicrobial drug resistance profiles have been used to identify and differentiate *E. coli* strains from different animal species (22). This type of testing is simple, cost-effective, and suitable for surveillance (45), and it has been used for *E. coli* strains collected from human and animal sources (22). Recently, multiple resistance profiles have been used to identify sources of fecal contamination in water (15, 16, 18, 19, 20, 30, 48, 49).

The use of antimicrobial agent resistance profiles to identify sources of bacterial contamination is a promising and emerging procedure. One technique that has been reported to be a useful, low-cost screening method is discriminant function analysis of antimicrobial agent resistance profiles (48). Unfortunately, little basic information is available for comparisons of the antimicrobial agent resistance profiles of normal gut microbiota from representative samples of domestic livestock and poultry, pets, wildlife, and humans simultaneously in the same geographic region. If the use of antimicrobial agents is an important factor for the development of antimicrobial agent resistance, it could be hypothesized that the patterns of antimicrobial agent resistance in different animal populations vary according to the types and quantities of agents used. To test this hypothesis, the two objectives of this study were (i) to identify patterns of antimicrobial agent resistance of *E. coli* strains obtained from human septage, domestic animals, and wildlife living in the Red Cedar watershed in Michigan, and (ii) to compare these antimicrobial agent resistance patterns with those of *E. coli* strains obtained from surface water in the same watershed.

MATERIALS AND METHODS

Study design. A repeated cross-sectional approach was used to collect samples and data related to antimicrobial agent use on farms over a 12-month period, from the winter of 2002 to the winter of 2003. Samples were collected every 3 months, and there were a total of four sampling periods during the study.

Study area. The sampling region was established by the boundaries of the Red Cedar watershed, from which surface water samples were obtained. This region encompasses an area of 1,186 km^2 in Ingham and Livingston counties in central Michigan. The Red Cedar River arises in Cedar Lake and flows approximately 73 km to its confluence with the Grand River in the city of Lansing. Swine and dairy cattle are the predominant forms of livestock in this watershed.

Enrollment of participating farmers. Farms were located within the Red Cedar watershed, and county drain commissioners identified specific farms whose premises drained into the watershed. The farmers were sent a letter through county extension agents, inviting them to participate in the study. Respondents returned a prestamped postcard to the Population Medicine Center at Michigan State University to indicate their willingness to participate. A total of 60 farmers were asked to participate in the study. Of 60 attempted contacts, 11 no longer maintained livestock and 5 had very few or no animals on their premises. A total of 31 of the remaining 44 farms agreed to participate, and farm visits were arranged quarterly from winter 2002 to winter 2003.

Sample size. In order to detect at least one animal with *E. coli* on a farm, the general formula used by Smith (42) was used to compute sample size (n_{inf}): $n_{inf} = \log(\alpha)/\log(1 - \text{prev})$, where α is the probability that none of the sampled animals harbor *E. coli* and prev is expected prevalence of *E. coli*.

We assumed that the expected prevalence of *E. coli* was 10% and that the type I error was 0.05. Using the equation and assumptions described above, we calculated that 29 animals per species was the minimum number necessary for testing.

Data collection. Data relating to antimicrobial agent use and numbers of animals on the farm were collected at the time of collection of fecal samples by using a questionnaire administered during an in-person interview. Participants were asked about the use of antimicrobial agents for therapy, prophylaxis, and growth promotion during the previous 60 days.

Sample collection. Fecal and farm environment samples were taken by using culturette swabs, and 100-ml water samples were collected from specific locations in the watershed. The water sampling sites were determined with the help of the Ingham county drain commissioner, based on the direction of the rain flow from every farm enrolled in the study. The water sampling bottles contained 10 mg of sodium thiosulfate to neutralize any residual chlorine in the water. All samples were shipped to the University of Maryland for bacterial isolation, identification, and antimicrobial agent susceptibility testing.

(i) Animal fecal samples. Fecal samples were obtained from dairy and beef cattle, swine, horses, sheep, goats, chickens, cats, dogs, deer, ducks, and geese. Fecal samples from livestock (dairy cattle, beef cattle, swine, sheep, goats, horses) and companion animals (dogs, cats) were collected rectally from individual animals by using culturette swabs. Samples were collected from fresh manure by using culturette swabs on feedlots where sampling of individual animals was not feasible. Poultry samples were collected by using cloacal swabs. Deer samples were collected from freshly voided droppings. Goose and duck samples were collected from freshly voided droppings and by using cloacal swabs by personnel from the Michigan Department of Natural Resources.

(ii) Farm environment samples. Samples from the manure storage facilities (lagoons, slurry pits, and manure piles) and animal housing areas on the farms were collected by using culturette swabs.

(iii) Septage samples. Samples representative of human fecal material were collected from septic tanks (prior to chemical treatment) with the help of the local septic pumping companies in the study area. Septage samples are the best representation of human-source fecal material that is likely to affect water and environmental quality via leakage from septic tanks or improper disposal of pumped septic contents in the study area.

Isolation of *E. coli* from water samples. The membrane filtration method used by the United States Environmental Protection Agency (46) was used to isolate *E. coli* from water samples. In this procedure, water samples were filtered through a sterile, white, grid-marked, 47-mm-diameter membrane (pore size, 0.45 ± 0.02 μm), which retained bacteria. After filtration, the membrane containing the bacteria was placed on a selective differential medium (mTEC agar) (9, 46) and incubated at 35°C for 2 h to resuscitate the injured or stressed bacteria and then at 44°C for 22 h. The filter was transferred from mTEC agar to a filter pad saturated with urea substrate medium. After 15 to 20 min, yellow, yellow-green, or yellow-brown colonies on mTEC agar were transferred to urea substrate media; any non-*E. coli* colonies turned pink or purple on these media.

Identification of *E. coli* from surface water, fecal, and human septage samples. Standard methods were used for the enrichment, isolation, identification, and biochemical confirmation of *E. coli* isolates (8).

Upon arrival at the laboratory, culturette swabs (fecal and human septage samples) or colonies picked from urea substrate media (surface water samples) were placed in tubes with tryptic soy broth (TSB) and incubated at 35°C for 24 h. Approximately 10 μl of the turbid broth was streaked onto violet red bile agar and incubated for 18 to 20 h at 35°C. The violet red bile agar plates were examined for reddish purple colonies that fluoresced under black light. Selected colonies were streaked onto MacConkey agar and incubated at 35°C for 18 to 20 h. The MacConkey agar plate was examined for red colonies that precipitated bile and had a dark red center. One or two colonies were selected and streaked onto tryptic soy agar and incubated for 18 h. The tryptic soy agar plate was then examined for single colonies that were round, milk colored, and slightly convex. A single colony was selected, placed in a tube containing TSB, and incubated for approximately 3 to 4 h until the culture was turbid.

Bacteria from the broth were transferred into tubes for biochemical confirmation by indole, methyl red, Voges-Proskauer, and Simmons citrate tests (8) on triple sugar iron (Difco, Sparks, Md.). Only the bacterial isolates that were confirmed to be *E. coli* based on the results of the biochemical tests were selected for antimicrobial agent sensitivity testing. Confirmed isolates were inoculated into new TSB tubes and incubated until the turbidity was 0.5 McFarland standard (approximately 2 to 3 h).

Antimicrobial agent susceptibility testing. Once a single *E. coli* isolate was isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of the *E. coli* isolates (27, 28) for 12 antimicrobial agents (Table 1). These antimicrobial agents were chosen on the basis of their importance in treating human or animal *E. coli* infections and their use as feed additives to promote growth in animals and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes (22).

A 150-mm Mueller-Hinton medium plate was swabbed with TSB inoculated with *E. coli* and incubated to a turbidity of 0.5 McFarland standard. Twelve commercially prepared antimicrobial agent disks were place on the inoculated plates. The plates were incubated at 35°C for 18 to 20 h. The diameters (in

TABLE 1. Concentrations and diffusion zone breakpoints for resistance for antimicrobial agents tested in this study, sorted by class of antimicrobial agent

Antimicrobial agent	Drug code	Disk drug concn (μg)	Diffusion zone breakpoint (mm)
Aminoglycosides			
Neomycin	N30	30	≤12
Gentamicin	GM10	10	≤12
Streptomycin	S10	10	≤11
Phinicols			
Chloramphenicol	C30	30	≤12
Quinolones and fluoroquinolones			
Ofloxacin	OFX5	5	≤12
Nalidixic acid	NA30	30	≤13
Sulfonamides and potentiated sulfonamides			
Sulfamethoxazole-trimethoprim	STX	23.75–1.25	≤10
Sulfisoxazole	G25	250	≤12
Tetracyclines			
Tetracycline	TE30	30	≤14
Beta-lactams			
Ampicillin	AM10	10	≤13
Nitrofurans			
Nitrofurantion	F/M 300	300	≤14
Cephalosporins			
Cephalothin	CF30	30	≤14

millimeters) of the clear zones of growth inhibition around the antimicrobial agent disks, including the 6-mm disk diameter, were measured by using precision calipers (27, 28). The breakpoints used to categorize isolates as resistant or not resistant to each antimicrobial agent were those recommended by the National Antimicrobial Resistance Monitoring System for *E. coli*. *E. coli* ATCC 25922 (American Type Culture Collection) was used for quality control.

Data analysis. Data for the antimicrobial agent resistance of each bacterial isolate were reported in two forms: either as the diameter of the zone of inhibition (in millimeters) or as resistant or not resistant (based on NCCLS breakpoints). Since these data were used to identify animal species sources of resistant *E. coli* in discriminant analysis, animal species were handled in two different ways, (i) individually by species and (ii) by groups based on animal management and the likelihood of exposure to various antimicrobial agents, as follows: livestock (cattle, pigs, sheep), wildlife (geese, ducks), and equines (horses, donkeys).

Associations between livestock group and antimicrobial agent resistance (resistant or not resistant) were expressed as odds ratios with 95% confidence intervals, and the Fisher exact test was used to test for significant differences between species groups (SAS 8.2; SAS Inc., Cary, N.C.). Differences in zones of inhibition between species groups were assessed by using analysis of variance and the nonparametric Wilcoxon rank sum χ^2 test (SAS 8.2; SAS Inc.).

RESULTS

A total of 31 farms agreed to participate in the study (Fig. 1), including 14 cattle farms (7 dairy farms and 7 beef farms), 6 sheep farms, 5 pig farms, 2 horse farms, 2 chicken farms, and 2 deer farms. Several farms had more than one species on the premises. A total number of 2,522 samples were collected, from which 1,286 *E. coli* isolates were retrieved for antimicrobial agent resistance profiling (Table 2). Data for use of antimicrobial agents, either alone or in combination with other drugs, were collected for 448 animals from 30 farms (Table 3). Overall, penicillin was the most commonly reported antimicrobial agent (86% overall), followed by chlortetracycline (30%), sulfamethazine (16%), and oxytetracycline (14%). The most widely used agents in food animals (dairy and beef cattle, swine, sheep) were chlortetracycline (dairy, beef, swine, sheep), oxytetracycline (dairy, beef, swine, sheep), and penicillin (dairy, beef, swine).

Antimicrobial agent resistance was detected in all types of samples collected (Table 4). The most frequently encountered form of resistance in all samples was resistance to tetracycline (27.3%), followed by resistance to cephalothin (22.7%), resistance to sulfisoxazole (13.3%), and resistance to streptomycin (13.1%). Animal fecal samples exhibited resistance to all agents tested, while human septage and river water samples showed resistance to three agents and one agent, respectively. Resistance to cephalothin was present in all types of samples, while tetracycline resistance and streptomycin resistance were found in all types of samples except river water. When we looked at the patterns of antimicrobial agent resistance for fecal, farm environment, and septage samples from different species groups (Table 5), *E. coli* strains from swine were resistant to 10 of the 12 agents tested (there was resistance in both fecal and farm environment samples from 9 of the 10 sources), followed by strains from dairy cattle, poultry, and beef cattle. Interestingly, when we compared resistance to tetracycline and resistance to trimethoprim-sulfamethoxazole in livestock isolates, we found that resistance to tetracycline was present in both fecal and farm environment samples from all livestock species, while resistance to trimethoprim-sulfamethoxazole was present in both types of samples from only dairy cattle and equids.

Disk diffusion zone sizes were also examined for differences between types of samples collected (Table 6). Significant differences were seen in the diffusion zone sizes for all agents except tetracycline and sulfisoxazole. Overall, the largest diffusion zones (indicating greater susceptibility) were found with animal fecal isolates. The exceptions were the diffusion zones for tetracycline, ampicillin, and sulfisoxazole; for these agents the water isolates had the largest diffusion zones. Human septage isolates had the smallest diffusion zones for all agents except neomycin, gentamicin, nitrofurantoin, and cephalothin, for which water isolates had the smallest zones.

Animal species were also used to examine patterns of resistance (Table 7). Since very little resistance to some of the agents was observed in this study, this analysis was limited to agents with the highest levels of resistance by type of sample: tetracycline, cephalothin, sulfisoxazole, streptomycin, and ampicillin. Based on the odds ratios for resistance, swine had the greatest likelihood of harboring *E. coli* resistant to tetracycline, sulfisoxazole, streptomycin, and ampicillin, while the lowest levels of resistance were seen with isolates from wild waterfowl and farmed deer (Table 7).

The patterns of antimicrobial agent resistance for isolates from farm environment samples were very similar to the patterns for isolates from animal fecal samples (Table 8). The only statistically significant difference found when we compared the rates of resistance to individual agents between all fecal samples and all farm environment samples combined was higher levels of resistance to cephalothin in farm environment samples (Table 8). When samples were classified by the species of animals living in the environment, significant differences were found. Swine fecal samples had higher levels of resistance to all

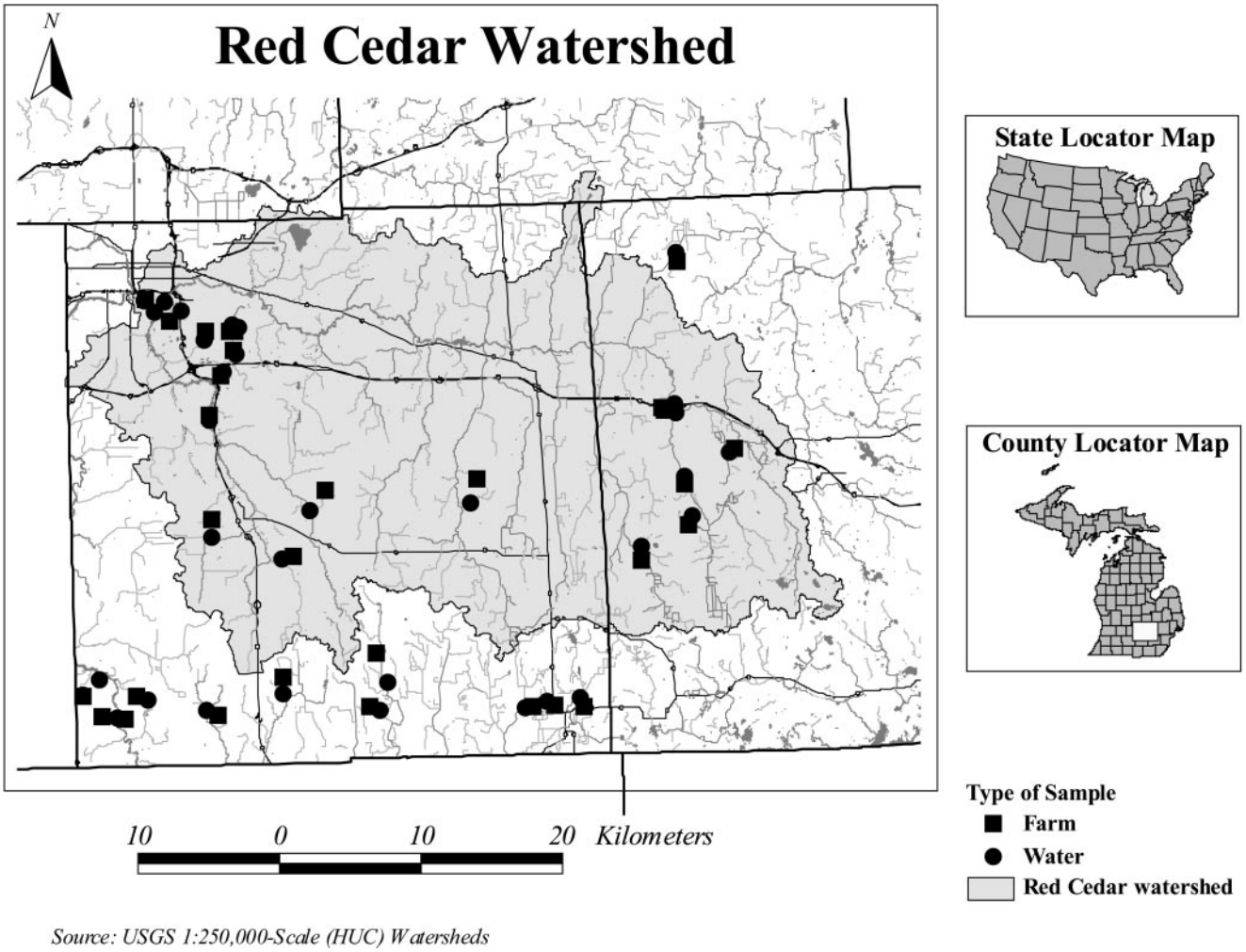

FIG. 1. Farm and surface water sampling locations in the Red Cedar watershed. The map was created by using USGS watershed data from the Michigan Center for Geographic Information (http://www.mcgi.state.mi.us/mgdl/?rel=thext&action=thmname&cid=3&cat=Watersheds).

of the antimicrobial agents except cephalothin. Cattle farm environment samples had significantly lower levels of resistance to tetracycline and sulfisoxazole than other farm environment samples. The patterns observed for disk diffusion zones for farm environment samples sorted by animal species were very similar to the patterns observed for levels of antimicrobial agent resistance; swine isolates showed reduced susceptibility to most drugs.

We also examined the patterns of antimicrobial agent resistance on farms with sufficient numbers of different animal species to determine whether there were any common patterns of resistance between species. Of the 31 farms used, 4 had sufficient numbers of different species (at least 10 species). Figure 2 shows the proportions of isolates with antimicrobial agent resistance for a farm that housed swine and poultry. As Fig. 2 shows, strains from both species showed high levels of resistance to tetracycline and streptomycin and similar levels of resistance to sulfisoxazole, and cephalothin.

Multidrug resistance was evaluated with *E. coli* isolates (Tables 9 and 10). The majority of *E. coli* isolates tested (52.33%) were sensitive to all antimicrobial agents tested, 34% were

TABLE 2. Samples collected and *E. coli* isolates recovered

Species	Fecal samples		Farm environment samples	
	No. collected	% with *E. coli*	No. collected	% with *E. coli*
Beef cattle	351	51.57	110	56.36
Dairy cattle	438	52.28	131	42.75
All cattle	789	51.96	118	48.96
Sheep	266	61.13	59	52.54
Goats	35	17.43	6	83.33
All small ruminants	301	59.77	65	55.38
Equids	120	50.0	53	32.08
Swine	327	54.43	93	40.86
Poultry	204	44.61	59	35.59
Farmed deer	60	56.67	NC[a]	
Humans	34	8.82	NC	
Companion animals	38	60.53	NC	
Wild geese	97	56.70	NC	

[a] NC, not collected.

TABLE 3. Antimicrobial agent use reported on farms expressed as percentages of animals receiving treatments for farms reporting treatments, by animal type

Antimicrobial agent	% of animals				
	Reported use (n = 438)	Dairy cattle (n = 131)	Beef cattle (n = 89)	Swine (n = 178)	Sheep (n = 17)
Aminoglycoside					
Streptomycin	9.60	32.82	0.0	0.0	0.0
Quinolones and fluoroquinolones					
Enrofloxacin	0.45	0.0	2.53	0.0	0.0
Sulfonamides and potentiated sulfonamides					
Sulfamethazine	16.07	20.61	55.70	0.0	0.0
Sulfamethoxazole-trimethoprim	0.22	0.76	0.0	0.0	0.0
Tetracyclines					
Tetracycline	13.92	12.66	0.0	0.0	0.0
Chlortetracycline	30.13	21.37	55.70	30.34	41.18
Oxytetracycline	14.29	16.79	16.46	8.99	58.82
Beta-lactams					
Penicillin	86.08	60.76	2.53	20.25	0.0
Ampicillin	0.89	3.05	0.0	0.0	0.0
Cloxacillin	0.67	2.29	0.0	0.0	0.0
Cephalosporins					
Ceftiofur	6.03	19.85	1.27	0.0	0.0
Macrolides					
Tilmicosin	8.70	0.76	27.85	8.43	0.0
Bacitracin					
Bacitracin	10.94	0.0	0.0	27.53	0.0

resistant to one or two antimicrobial agents, and 13% were resistant to three or more agents. The highest levels of multidrug resistance were found in swine, and no multidrug resistance was seen in farmed deer or wild geese (Table 9). The majority of multidrug resistance combinations included tetracycline resistance (Table 10). The combination tetracycline resistance and sulfamethazine resistance was found in 12% of all isolates and in more than one-half of all multidrug-resistant isolates (Table 10).

DISCUSSION

Similar patterns of resistance of *E. coli* were found for animal fecal and farm environment samples classified by animal species, suggesting that there were common sources of resistant bacteria (Table 4). Livestock functioned as a reservoir of resistant bacteria for environmental contamination, particularly in cases where higher levels of resistance were seen in fecal isolates than in farm environment isolates (all antimicro-

TABLE 4. Percentages of isolates exhibiting antimicrobial agent resistance, by type of sample

Antimicrobial agent	% of isolates				
	Animal fecal (n = 1,037)	Farm environment (n = 230)	Human septage (n = 3)	Surface water (n = 26)	Overall (n = 2,552)
Neomycin	4.72	3.91	0.0	0.0	4.51
Gentamicin	0.77	1.30	0.0	0.0	0.86
Streptomycin	13.21	13.04	33.33	0.0	13.06
Chloramphenicol	1.06	1.30	0.0	0.0	1.09
Ofloxacin	0.19	0.0	0.0	0.0	0.16
Sulfamethoxazole-trimethoprim	2.22	3.47	0.0	0.0	2.41
Tetracycline	28.06	25.65	33.33	0.0	27.29
Ampicillin	5.59	4.35	0.0	0.0	5.29
Nalidixic acid	0.67	0.0	0.0	0.0	0.54
Nitrofuratoin	0.87	0.43	0.0	0.0	0.78
Cephalothin	20.54	30.43	33.33	80.6	22.71
Sulfisoxazole	13.98	11.30	0.0	0.0	13.30

TABLE 5. Patterns of antimicrobial agent resistance in *E. coli* isolated from various sources (feces, environment, septage)

Antimicrobial agent	Dairy cattle	Beef cattle	Swine	Poultry	Small ruminants	Equids	Farmed deer	Wild geese	Companion animals	Human septage
Neomycin	R[a]	r	R	R	r	S	S	S	S	S
Gentamicin	r	r	R	R	r	R	S	S	S	S
Streptomycin	R	R	R	R	R	R	S	S	R	R
Chloramphenicol	r	R	R	R	R	S	S	S	S	S
Ofloxacin	S	S	R	S	S	S	S	S	S	S
Sulfamethoxazole-trimethoprim	R	r	r	r	r	R	S	S	S	S
Tetracycline	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	r	r	R	S	S	R	S
Nalidixic acid	S	S	S	r	r	S	S	S	S	S
Nitrofurantoin	R	R	S	r	r	S	S	S	S	S
Cephalothin	R	R	R	R	R	r	R	R	R	R
Sulfisoxazole	R	R	R	R	R	R	S	S	S	S

[a] R, *E. coli* isolates from fecal, farm environment, and septage samples all exhibited resistance; r, *E. coli* isolates from either fecal, farm environment, or septage samples exhibited resistance; S, all *E. coli* isolates from fecal, farm environment, or septage samples exhibited susceptibility.

bial agents except cephalothin, chloramphenicol, gentamicin, and trimethoprim-sulfamethoxazole) (Table 4). The similarities of the patterns of resistance seen in different species from the same farm (Fig. 2) also suggested that there was a common source of resistant bacteria for the different species. While the patterns of resistance on the farm described in Fig. 2 were not identical for swine and poultry isolates, the high levels of tetracycline resistance (>80% for both species groups) and the prominence of streptomycin, cephalothin, and ampicillin resistance suggest that there was a common source of resistance. The differences in resistance patterns may have been due to exposure to different agents because of differences in the husbandry of these species or other factors that may have increased or decreased the likelihood of the development and conservation of resistant bacteria in the animal species.

The differences in antimicrobial agent resistance patterns can be used to differentiate sources of fecal contamination in water with analytical tools such as discriminant function analysis (15, 16, 18–20, 30, 48, 49). Ribotyping of *E. coli* isolates has been suggested for use in discriminant function analysis for determination of fecal pollution sources (3, 5, 31), as genetic profiles are less susceptible to localized selection pressures than antimicrobial agent resistance patterns are (31), which may make decision rules developed with these data more useful on a broader geographic and temporal scale. While molecular techniques such as repetitive extragenic palindromic PCR or RNA ribotyping provide definitive means of source identification, there are several advantages to the utilization of antimicrobial agent resistance profiles as an alternative means of source determination. The facilities and expertise required to obtain isolation and antibiotic sensitivity profiles are available in most bacteriology laboratories, while genetic fingerprinting techniques for microbial source tracking are more expensive and require facilities with appropriate equipment and expertise.

Interestingly, farm environment isolates showed reduced susceptibility (as measured by disk diffusion zone sizes) compared to fecal sample isolates for all agents except sulfisoxazole (Table 6). This may indicate that there were sources of resistance factors that were not sampled in this study, such as farm workers, other domestic animals, and wildlife with access to the farm environment. It has been demonstrated that bacteria in the soil can acquire resistance to tetracycline from environmental exposure, possibly creating a reservoir of resistance factors generated outside host animals (35). This finding also suggests that, while collection of environmental samples from a farm may not be a valid means of assessing the prevalence and distribution of antimicrobial agent resistance patterns in

TABLE 6. Mean disk diffusion zones and resistance breakpoints for *E. coli* isolates, by type of sample

Antimicrobial agent	Breakpoint (mm)	Mean disk diffusion zone diam (mm)				Wilcoxon rank sum *P* value[a]
		Animal fecal (n = 1,037)	Farm environment (n = 230)	Human septage (n = 3)	Surface water (n = 26)	
Neomycin	≤12	17.68	17.10	15.93	15.66	0.0140
Gentamicin	≤12	21.03	20.13	18.36	18.16	0.0001
Streptomycin	≤11	15.27	14.54	12.33	13.45	0.0022
Chloramphenicol	≤12	25.61	25.01	22.70	24.41	0.0062
Ofloxacin	≤12	29.36	28.43	25.43	27.40	0.0052
Sulfamethoxazole-trimethoprim	≤10	26.84	25.74	24.90	24.99	0.0330
Tetracycline	≤14	18.82	18.79	15.47	21.89	0.4588
Ampicillin	≤14	19.31	18.86	15.47	21.89	0.0217
Nalidixic acid	≤13	24.51	23.41	21.20	22.53	0.0098
Nitrofuratoin	≤14	21.09	20.36	18.30	17.56	<0.0001
Cephalothin	≤14	17.78	16.93	14.33	13.81	<0.0001
Sulfisoxazole	≤12	20.68	20.95	20.60	22.08	0.5049

[a] Test for significant differences in disk diffusion zones between types of samples.

TABLE 7. Percentages of fecal isolates resistant to the five agents with most commonly reported resistance and odds ratios for resistance, by species group

Species group	*n*	% of fecal isolates resistant (mean odds ratio; odds ratio range)				
		Tetracycline	Cephalothin	Sulfisoxazole	Streptomycin	Ampicillin
Cattle	407	23.10 (0.65; 0.49 – 0.87)	21.87 (1.13; 0.83 – 1.54)	9.74 (0.52; 0.35 – 0.77)	10.20 (0.62; 0.42 – 0.92)	2.53 (0.30; 0.15 – 0.60)
Swine	168	63.10 (6.28; 4.41 – 8.93)	17.26 (0.77; 0.50 – 1.19)	35.84 (5.09; 3.47 – 7.48)	30.59 (3.98; 2.68 – 5.91)	18.79 (6.96; 4.02 – 12.03)
Poultry	97	35.05 (1.42; 0.92 – 2.21)	20.62 (1.00; 0.60 – 1.67)	16.67 (1.22; 0.69 – 2.16)	15.05 (1.16; 0.64 – 2.12)	2.06 (0.32; 0.08 – 1.33)
Small ruminants	181	24.31 (0.79; 0.54 – 1.14)	22.10 (1.11; 0.75 – 1.64)	11.11 (0.71; 0.43 – 1.17)	12.22 (0.88; 0.54 – 1.44)	5.23 (0.88; 0.43 – 1.83)
Equids	61	9.84 (0.26; 0.11 – 0.62)	21.31 (1.04; 0.55 – 1.96)	11.86 (0.80; 0.35 – 1.79)	10.00 (0.71; 0.30 – 1.67)	7.14 (1.27; 0.44 – 3.65)
Companion animals	21	9.52 (0.26; 0.06 – 1.14)	38.10 (2.41; 0.99 – 5.92)	4.35 (0.27; 0.04 – 2.00)	9.09 (0.64; 0.15 – 2.78)	4.17 (0.70; 0.09–5.31)
Farmed deer	34	2.94 (0.07; 0.01 – 0.54)	11.76 (0.50; 0.18 – 1.44)	0.0 (0.85; 0.83 – 0.87)	0.0 (0.86; 0.84 – 0.88)	0.0 (0.94; 0.93 – 0.96)
Wild waterfowl	54	1.85 (0.04; 0.01 – 0.33)	11.11 (0.47; 0.20 – 1.10)	0.0 (0.05; 0.003 – 0.89)	0.0 (0.06; 0.003 – 0.90)	0.0 (0.94; 0.92 – 0.95)

TABLE 8. Percentages of farm environment sample isolates resistant to the five agents with most commonly reported resistance and odds ratios for resistance, by species group

Manure source	*n*	% of farm environment sample isolates resistant (mean odds ratio; odds ratio range)				
		Tetracycline	Cephalothin	Sulfisoxazole	Streptomycin	Ampicillin
Cattle	126	15.87 (0.38; 0.21 – 0.71)	30.95 (1.26; 0.72 – 2.20)	4.24 (0.20; 0.07 – 0.55)	9.84 (0.61; 0.28 – 1.32)	1.64 (0.24; 0.05 – 1.15)
Swine	38	55.26 (5.46; 2.63 – 11.33)	36.84 (1.56; 0.76 – 3.23)	26.32 (4.0; 1.65 – 9.68)	27.50 (3.61; 1.56 – 8.37)	17.50 (14.28; 3.51 – 58.02)
Small ruminants	37	18.92 (0.70; 0.29 – 1.68)	21.62 (0.65; 0.28 – 1.49)	16.67 (1.77; 0.66 – 4.77)	11.11 (0.86; 0.28 – 2.62)	0 (0.25; 0.01 – 4.39)
Poultry	23	34.78 (1.78; 0.71 – 4.43)	21.74 (0.67; 0.24 – 1.87)	8.0 (0.67; 0.15 – 3.01)	0 (0.12; 0.01 – 2.01)	0 (0.38; 0.02 – 6.61)
Equids	20	15.0 (0.53; 0.15 – 1.87)	20.0 (0.60; 0.19 – 1.86)	18.75 (1.95; 0.52 – 7.34)	17.65 (1.56; 0.42 – 5.77)	5.00 (1.26; 0.15 – 10.51)
All farm environment samples[a]	244	24.18 (0.83; 0.60 – 1.14)	28.69 (1.50; 1.10 – 2.06)	11.16 (0.77; 0.49 – 1.20)	12.50 (0.94; 0.61 – 1.43)	4.08 (0.71; 0.36 – 1.41)

[a] Test for differences between farm environment samples and other types of samples (fecal, septage, water).

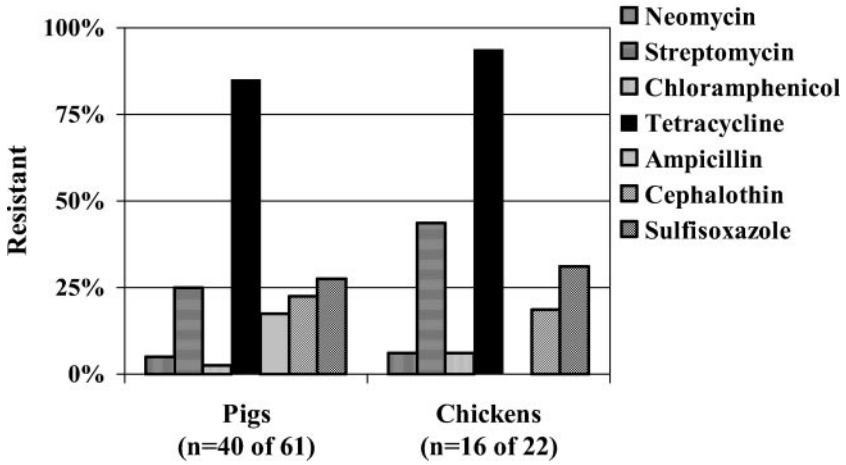

FIG. 2. Antimicrobial resistance of fecal *E. coli* isolates from different species residing on the same farm.

animals residing on the farm, it may be a more accurate measure of exposure to resistance factors from farm runoff in watersheds. Additional research is needed to address this question, including expanding the collection of samples to other potential host sources of resistant bacteria and comparing the genetic characteristics of bacteria surviving in the farm environment to the genetic characteristics of bacteria isolated from uncontaminated specimens taken per rectum and obtained from farm runoff

The highest levels of resistance (reduced susceptibility) were observed for tetracycline and cephalothin with isolates collected from all types of samples (Table 4) and animal species groups. The presence of tetracycline and cephalothin resistance in *E. coli* from a variety of sources agrees with findings of other studies on the antimicrobial agent resistance of *E. coli* from a variety of different sources throughout the world (11, 21, 38, 40).

The patterns of resistance to the antimicrobial agents may be due to widespread and lengthy use of tetracycline and cephalosporin. Since both tetracycline and cephalosporins are naturally derived compounds, bacteria can be exposed to these agents in nature and outside any human use for disease treatment, for prophylaxis, or for livestock growth promotion. Tetracycline is a commonly used first-line antibiotic for many different species of domestic animals and is often used before the antimicrobial agent resistance of a pathogen has been determined (33). Cephalothin is labeled for use in nonruminants and is not used with food animals. However, the third-generation cephalosporin ceftiofur is commonly used for mastitis prevention and treatment in dairy cows, and resistance that develops against ceftiofur can result in resistance to first-generation cephalosporins, such as cephalothin.

Resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants, while resistance to many of the cephalosporins is often the result of stable mutations (33). Plasmid-mediated acquired resistance to third-generation cephalosporins has also been reported (32). The large numbers of genetic determinants for tetracycline resistance make it more possible for a susceptible bacterium to acquire resistance factors than if only a few determinants were available. The stable mutations which confer resistance to cephalosporins are easily retained by bacteria, even in the absence of selective pressure to maintain resistance.

The lowest levels of resistance (increased susceptibility) found in this study were the levels of resistance to ofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole (Tables 4 to 6). These antimicrobial agents are members of drug classes that have restricted uses in veterinary medicine. The use of fluoroquinolones (ofloxacin and nalidixic acid) has been restricted since the 1990s, after the rapid emergence of resistance to fluoroquinolones after the introduction of enrofloxacin into poultry production in Europe (10). Chromosomal mutations

TABLE 9. Percentages of multidrug-resistant *E. coli* isolates, by species group

Source	*n*	% of isolates resistant to:					Mantel Haensel χ^2 *P* value
		No agent	One agent	Two agents	Three agents	More than three agents	
All	1,286	52.33	26.05	8.48	5.91	7.23	
Dairy cattle	229	60.26	27.51	3.49	2.62	6.11	0.0017
Beef cattle	176	53.41	27.27	9.09	3.98	6.25	0.7703
Small ruminants	184	59.24	20.65	8.15	7.61	4.35	0.0954
Swine	178	17.42	30.90	20.79	16.29	14.61	<0.0001
Poultry	90	44.44	27.78	11.11	4.44	12.22	0.2199
Equids	60	68.33	16.67	1.67	3.33	10.00	0.0402
Farmed deer	34	91.18	8.82	0	0	0	0.0002
Companion animals	23	65.22	21.74	8.70	0	4.35	0.6335
Wild geese	55	85.45	14.55	0	0	0	<0.0001

TABLE 10. Most commonly identified combinations of antimicrobial agents in multidrug-resistant isolates from all sources

Antimicrobial agent combination	% of:	
	All isolates	Multidrug-resistant isolates
Tetracycline-sulfamethazine	12.01	55.96
Tetracycline-streptomycin	10.89	50.54
Streptomycin-sulfamethazine	8.64	40.07
Tetracycline-cephalothin	6.38	29.00
Tetracycline-ampicillin	5.29	24.55
Tetracycline-sulfamethazine-streptomycin[a]	7.94	36.82

[a] Includes the data for the tetracycline-sulfamethazine, tetracycline-streptomycin, and streptomycin-sulfamethazine determinations.

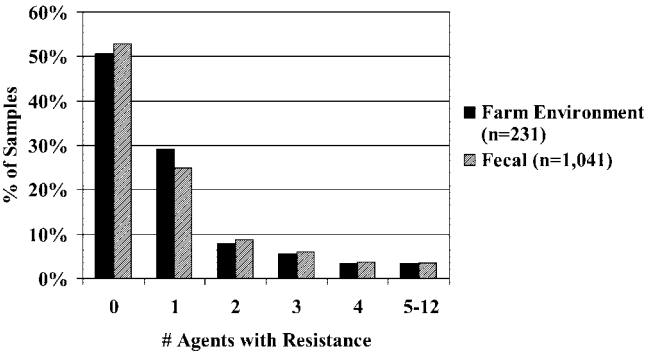

FIG. 3. Multidrug resistance of *E. coli* isolates, sorted by type of sample.

confer resistance to fluoroquinolones (33), and the development of resistance to one agent results in cross-resistance to other fluoroquinolones. In the United States, fluoroquinolone use is prohibited in food animals except for the treatment of acute pneumonia in beef cattle, and currently the Center for Veterinary Medicine of the U.S. Food and Drug Administration is working to ban the use of enrofloxacin in poultry production in the United States (7). In addition to restrictions on their use, fluoroquinolones were introduced into clinical medicine only 20 years ago, making them relatively new antimicrobial agents, and animal populations do not have a long history of exposure to these drugs compared to the history of exposure to other agents, such penicillin or tetracycline.

The use of sulfonamides was restricted for food animals in the 1980s after a potential threat to human health from residues in foods of animal origin, and they are currently approved for use in treating calf scours. These drugs are broad-spectrum antimicrobial agents with a history of more than 50 years of veterinary use. Resistance in sulfonamides is plasmid mediated, but chromosomal mutations for sulfonamide resistance take place very slowly (33). Since resistance to sulfonamides is widespread and cross-resistance between sulfonamides is complete, they are considered to be of limited use in treatment of ruminants (33). Sulfonamide use for growth promotion in swine is controversial because of persistent problems with violative residues in swine carcasses (12, 33).

As hypothesized, the highest levels of resistant *E. coli* isolates were obtained from food animals (cattle, swine, poultry, small ruminants), followed by companion animals, equids, and farmed deer. Wildlife, which are not intentionally exposed to large quantities of antimicrobial agents, exhibited a significantly lower prevalence of antimicrobial agent resistance. This is consistent with the hypothesis that exposure of commensal gut microbiota to antimicrobial agents selects for resistant bacterial strains (33, 45). When species exposure groups were examined, swine had the highest levels of resistance to all antimicrobial agents (Table 5) for fecal (Table 7) and farm environment (Table 8) isolates and had the highest levels of multidrug-resistant isolates (Table 9). Poultry had the next highest level of antimicrobial agent resistance. Because in this study we were not able to collect detailed data on the use of antimicrobial agents from several of the producers, we did not specifically assess the association between administration of antimicrobial agents and subsequent recovery of resistant isolates from individual animals.

The use of antimicrobial agents for swine and poultry production may be associated with these trends. There is widespread use of antimicrobial growth promoters in swine (arsenicals, bacitracin, bambermycins, carbadox, tetracycline, chlortetracycline-sulfamethazine-penicillin, lincomycin, penicillin, tiamulin, tylosin, virginiamycin) and poultry (arsenicals, bacitracin, bambermycins, tetracycline, lincomycin, penicillin, virginiamycin) (41). Antimicrobial agents are also provided in water to prevent diseases in poultry flocks and in milk replacers to prevent diseases in calves. The antimicrobial agent classes that could be used for growth promotion in this study included tetracycline for swine, poultry, and cattle and beta-lactams (ampicillin) for swine and poultry. The livestock species that are routinely exposed for extended periods to subtherapeutic doses of antimicrobial agents (swine, poultry, cattle) exhibited a significantly higher prevalence of resistance than the species that are typically only exposed to therapeutic doses for brief periods (47). In addition to antimicrobial agent use for growth promotion or disease prevention, the use of these agents for disease treatment, in both recommended and extralabel uses, contributes to the exposure of enteric bacteria in affected hosts.

Little resistance (reduced susceptibility) was seen in wild geese in this study. When wildlife species are considered as sources of resistant bacteria or sentinels for the spread of resistant organisms from the farm to surrounding ecosystems, wild Canada geese (*Branta canadensis*) may be good indicators of potential exposure of a local area to outside resistance factors but poor indicators of local source exposure. Canada geese are migratory birds with summer and winter ranges that are considerable distances apart, and it is difficult to determine where an animal was exposed to a resistant bacterium. To assess the spread of resistance from the farm to local wildlife, wildlife species with limited home ranges or migration patterns would make better sentinel species than wild geese.

Multidrug resistance was found in *E. coli* isolated from all domestic species (Table 9) and from both fecal and farm environment samples (Fig. 3), and the highest levels were in food animals compared to other animal species (Table 9). More than 21% of all *E. coli* isolates in this study exhibited resistance to more than one agent. When different species were examined, the groups with the highest levels of multidrug-resistant

E. coli were swine, poultry, and ruminants. When fecal and farm environment samples were compared, the similarity in the levels of multidrug-resistant isolates for different types of samples reinforced the concept that they had common sources of resistant bacteria (Fig. 3). Most multidrug-resistant isolates exhibited resistance to a combination of antimicrobial agents that included tetracycline, which may suggest that *E. coli* strains that are tetracycline resistant are also at increased risk for becoming resistant to additional antimicrobial agents. Resistance to tetracycline may be conserved in bacterial populations over time, regardless of selection pressure, which might result in an overall increase in resistance over time. Additionally, the multidrug resistance exhibited by *E. coli* in this study could have been the result of independent, simultaneous development of resistance to different agents or could have been the result of coselection of resistance determinants. Studies have demonstrated that in *Salmonella*, cephalosporin resistance is cotransferred with additional resistance markers for chloramphenicol, sulfamethoxazole, and tetracycline (34) and that transfer of the plasmid containing the gene, designated CMY-2, between *Salmonella* and *E. coli* isolates from food animals and humans has been found (50). Given this situation, exposing a bacterial population to one antimicrobial agent may result in resistance to other agents without any prior exposure.

Conclusions. The results of this study showed that antimicrobial agent resistance was present in *E. coli* strains isolated from a variety of domestic animal species and farm environments and that the resistance varied depending on the type of sample and species. Organisms appeared to show higher levels of resistance or reduced susceptibility to some specific antimicrobial agents in farm environments (outside animal hosts). This may have been the result of resistance factors that are readily retained by *E. coli*, the easy acquisition of resistance factors outside the host, or significant sources of resistant bacteria not captured by this study. The collection of more bacterial isolates from different sources and the addition of genetic analysis should provide more information on the dynamics of the introduction and spread of resistant bacteria on the farm. The ultimate goal of this research was to identify sources of fecal contamination of surface waters. Given that the patterns of antimicrobial agent resistance in this study were similar for fecal and farm environment samples, regardless of the source species, restricting sampling to the farm environment may be a desirable approach when resources are limited. This study showed that the results of antimicrobial agent susceptibility testing for farm environment samples were similar to the results for fecal samples, which could reduce the number of samples required at sampling specific locations on the farm rather than individual animals. This should also make sampling easier in that animal handling should not be necessary. Finally, results from farm environments may be more representative of the actual exposure to surface water, compared to uncontaminated bacteria from individual animals.

This study showed the distribution of antimicrobial agent resistance in *E. coli* isolates from a variety of sources, and analysis of such patterns of resistance may prove to be useful beyond simple description. As concerns about water quality and environmental contamination by human and agricultural waster have increased, it has become increasingly important to develop low-cost screening tools that can be used to identify the most probable source of fecal contamination. The distinct patterns of antimicrobial agent resistance may prove to be a valuable tool for the development of multivariate statistical techniques for bacterial source identification.

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