Comparison of clonal relatedness and antimicrobial susceptibility of fecal *Escherichia coli* from healthy dogs and their owners

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Objective—To determine prevalence of within-household sharing of fecal *Escherichia coli* between dogs and their owners on the basis of pulsed-field gel electrophoresis (PFGE), compare antimicrobial susceptibility between isolates from dogs and their owners, and evaluate epidemiologic features of cross-species sharing by use of a questionnaire.

Sample Population—61 healthy dog-owner pairs and 30 healthy control humans.

Procedures—3 fecal *E coli* colonies were isolated from each participant; PFGE profiles were used to establish relatedness among bacterial isolates. Susceptibility to 17 antimicrobials was determined via disk diffusion. A questionnaire was used to evaluate signalment, previous antimicrobial therapy, hygiene, and relationship with dog.

Results—A wide array of PFGE profiles was observed in *E coli* isolates from all participants. Within-household sharing occurred with 9.8% prevalence, and across-household sharing occurred with 0.3% prevalence. No behaviors were associated with increased clonal sharing between dog and owner. No differences were found in susceptibility results between dog-owner pairs. Control isolates were more likely than canine isolates to be resistant to ampicillin and trimethoprim-sulfamethoxazole. Owners and control humans carried more multdrug-resistant *E coli* than did dogs.

Conclusions and Clinical Relevance—Within-household sharing of *E coli* was detected more commonly than across-household sharing, but both direct contact and environmental reservoirs may be routes of cross-species sharing of bacteria and genes for resistance. Cross-species bacterial sharing is a potential public health concern, and good hygiene is recommended. (*Am J Vet Res* 2009;70:1108–1116)

A strong human-animal bond exists for companion animals, with up to 99% of dog and cat owners considering their pets to be part of their family.[1] Close relationships between dogs and their owners raise public health concerns regarding transmission of infectious organisms between species.[2] Dogs have been considered a potential reservoir for *Escherichia coli* capable of causing urinary tract infections in women on the basis of studies[3,4] revealing similarities in PFGE profiles and virulence factor patterns among canine fecal *E coli* isolates and *E coli* isolates from women with cystitis, pyelonephritis, or urosepsis. On the contrary, a comparison of antimicrobial susceptibility of canine fecal and human fecal and urine *E coli* found the lowest percentage resistance in canine isolates and concluded that dogs may be at risk for acquiring resistant *E coli* strains from humans.[5] Although these studies suggest cross-species bacterial sharing may occur, both studies isolated *E coli* from dogs and humans with no known direct or environmental contact.

The public health implications of the canine reservoir hypothesis warrant further investigation into the prevalence of *E coli* sharing between dogs and their owners. Within-household sharing of clonal fecal *E coli*

Abbreviations

MDR	Multidrug resistant
MUG	4-methylumbelliferyl-β-D-glucuronide
PFGE	Pulsed-field gel electrophoresis

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has been verified in humans and their pets in 2 longitudinal case studies.[6,7] Concurrent with the study reported here, Johnson et al[8] performed a study determining prevalence of within-household cross-species sharing of *E coli* with a focus on households with acute urinary tract infections by use of random-amplified polymorphic DNA analysis and PFGE.

The objectives of the study reported here were to determine prevalence of within-household sharing of fecal *E coli* between healthy dogs and their owners by use of PFGE, compare antimicrobial susceptibility patterns between isolates from dog and owner, and study the epidemiologic features of cross-species *E coli* sharing by use of a questionnaire. Sharing was defined as isolation of an *E coli* clone with ≥ 94% PFGE similarity from 2 individuals; we did not attempt to determine transmission or direction of transmission. We hypothesized that dogs and owners within households would have fecal *E coli* isolates with shared PFGE profiles and similar susceptibility results, suggesting that transmission may occur in either direction.

Materials and Methods

Study design and participants—A cross-sectional study of fecal *E coli* isolates from 61 pairs of dogs and their owners and 30 control humans was performed. A sample size of 61 dog-owner pairs was calculated for a McNemar χ^2 analysis with $\alpha = 0.05$, effect size of 0.30, and 98% power. All human participants were healthy and > 18 years of age. Dogs and owners lived in the same household for at least 6 months, and only 1 dog and owner per household participated. No human or canine participants received antimicrobial therapy within 2 weeks of enrollment. Controls did not own a dog or cat and did not have > 1 hour of contact/wk with a dog. Recruitment was from the faculty, staff, and student body of the University of Tennessee College of Veterinary Medicine, other departments within the University of Tennessee, and a private local business.

Compliance—This study was granted approval by both the Institutional Review Board and Institutional Animal Care and Use Committee of the University of Tennessee to ensure appropriate treatment of all participants. All human participants were informed of potential risks and benefits of participation as well as confidentiality and signed a standardized informed consent form.

Sample collection—Swabs[a] in transport medium were provided, and participants were instructed to collect a fecal swab sample from themselves and their dog. Paired samples from owners and dogs were collected during the same 12-hour period and submitted together. Each human participant answered a questionnaire, which was developed in a focus group of dog owners and was evaluated in a preliminary study prior to use. Questions were designed to identify demographics, signalment, medication history, hygiene, and relationship between dog and owner.

***E coli* isolation**—Each fecal swab specimen was diluted in peptone water, and a 20-µL sample was streaked for isolation on each of 2 plates containing either eosin methylene blue agar with 0.1 g of MUG fluorescence crystals[b]/L or a different selective medium with MUG.[c] The MUG was used as a differential agent to identify presumptive *E coli* colonies; most strains of *E coli* possess β-glucuronidase, which hydrolyzes MUG to 4-methylumbelliferone, causing colonies to fluoresce blue under 366-nm UV light. Plates were incubated at 44°C for 18 to 24 hours and examined under UV light. Three presumptive *E coli* colonies were each separately inoculated into 5 mL of brain-heart infusion broth and incubated at 44°C for 18 to 24 hours. After incubation, 20 µL was streaked for isolation. Plates were incubated at 44°C for 18 to 24 hours and examined under UV light. Presumptive *E coli* colonies were confirmed with biochemical test kits.[d] Pure confirmed *E coli* isolates were stored in brain-heart infusion broth with 10% glycerol at –85°C until further use.

PFGE—Pulsed-field gel electrophoresis was performed on 2 *E coli* isolates chosen arbitrarily from each participant. Preparation of DNA and PFGE procedures were performed as described with minor modifications.[9] Isolates were incubated overnight in Luria-Bertani broth at 35°C. Overnight cultures were concentrated and washed in PBS solution. Protease K[e] (25 µL) was added to each *E coli* sample. Two percent agarose gel was prepared by combining 1 g of pulse field certified agarose with 50 mL of 1X Tris-EDTA buffer. Equal amounts of *E coli* sample and 2% agarose gel were added to disposable molds. Lysis buffer contained 2.25 mL of 1M Tris HCl, 4.5 mL of 0.5M EDTA, 4.5 mL of 10% sarcosine,[f] 225 µL of protease K,[e] and 18.5 mL of sterile distilled water. Plugs in lysis solution were incubated in a 54°C water bath overnight.

After incubation, DNA-containing plugs were washed in Tris-EDTA buffer. Plugs were incubated in 200 µL of prerestriction buffer containing 20 µL of 10X buffer D[g] and 180 µL of nuclease-free water at 21°C for 5 minutes. Plugs were digested overnight in a restriction enzyme solution containing 40 µL of 10X buffer D,[g] 5 µL of bovine serum albumin[h] (10 mg/mL), 5 µL of restriction endonuclease XbaI,[i] and 351 µL of nuclease-free water in a 37°C water bath.

Electrophoresis was performed with a standard PFGE unit[j] by use of 0.5X Tris-borate EDTA buffer solution. The DNA fragments were resolved on 1% agarose gel. Plugs from paired dog and owner isolates were analyzed on the same gel to minimize effects of gel-to-gel variability on interpretation of similarity between paired isolates. Low-range pulse field gel markers[k] were loaded into wells 1, 8, and 15 of each gel. Pulse times ramped from 6 to 40 seconds during a 24-hour run at 6 V/cm, with a cooling temperature of 14°C. Gels were stained with ethidium bromide (1 µL/400 mL of water) for 30 minutes, destained with water for 75 minutes, and photographed.

Antimicrobial susceptibility testing—*Escherichia coli* isolates were tested for susceptibility to 17 antimicrobials by use of the disk diffusion method. Standard antimicrobial disk potencies were used, including amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftiofur (30 µg),

cefpodoxime (10 µg), ceftriaxone (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (30 µg). Routine quality control of antimicrobial disks was performed prior to and throughout the study for new lots of disks or Mueller-Hinton agar by use of *E coli* American Type Culture Collection 25922 and *Staphylococcus aureus* American Type Culture Collection 25923. Diameters of zones of inhibition were interpreted in accordance with current guidelines of the Clinical and Laboratory Standards Institute, formerly known as the National Committee for Clinical Laboratory Standards.[10–12] Multiple drug resistance was defined as resistance to 3 or more antimicrobials, regardless of class.

Statistical analysis—Descriptive statistics were used to summarize demographic and signalment data, medication history, hand washing, and behaviors; *t* tests were used to compare independent interval data. A commercial software program[l] was used to analyze bands from electrophoresis gels and to generate similarity indices among *E coli* isolates. The unweighted-pair group method with averaging based on Dice similarity coefficients and a clustering algorithm was used to create dendrograms. One percent optimization and 2% position tolerance were used. *Escherichia coli* isolates with ≥ 94% PFGE similarly were considered shared clones. Total potential clone-sharing pairs was calculated as the overall number of pairwise combinations of participants (n[n – 1]/2, where n is the total number of participants).[8] Number of potential across-household sharing pairs was calculated as the difference between total potential clone-sharing pairs and within-household sharing pairs. A χ^2 analysis was used to compare within-household versus across-household sharing, and χ^2, Mann-Whitney U, and Kruskal-Wallis analyses were used to compare demographics and dog-owner behaviors with within-household percentage PFGE similarity. A value of $P \leq 0.05$ was considered significant.

The Wilcoxon signed rank test was performed to compare susceptibility results between paired dogs and owners. Data were also collapsed, converting intermediate to resistant results, and McNemar χ^2 tests were used to compare paired dog and owner susceptibility results. Mann-Whitney tests were used to compare susceptibility results as ordinal data, between independent dogs and controls, and between owners and controls. Mann-Whitney and χ^2 analyses were used to compare susceptibility results with variables such as history of antimicrobial therapy and hand-washing behaviors. A commercial statistical software program[m] was used to compute all statistics. The original threshold for significance was set at a value of $P \leq 0.05$. The Bonferroni correction for multiple comparisons (17 antimicrobial agents) was applied, lowering the threshold for significance to a value of $P \leq 0.003$.

Results

Demographics and signalment—There was no difference in age ($P = 0.168$) or gender distribution ($P = 0.133$) between owners and controls. Of the 91 human participants, 41 of 61 (67.2%) owners and 18 of 30 (60.0%) controls were recruited from the University of Tennessee College of Veterinary Medicine ($P = 0.317$). Dog ages ranged from 6 months to 18 years. There were 29 spayed females, 6 sexually intact females, 24 neutered males, and 6 sexually intact males. Thirty-nine percent of dogs were mixed-breed dogs, and 23 breeds of purebred dogs were represented. Forty-one percent of dogs were acquired from breeders, 39.3% from shelters or as strays, 3.3% from pet stores, and 16.4% from other sources (eg, family or friends). Eighty-two percent of dogs lived in multi-pet households.

Questionnaire results—Seventy percent of owners allowed their dogs to lick or kiss them on the face, 54.1% of owners spent > 30 h/wk of awake time with their dog, 54.1% allowed the dog to sleep in their bed with them, and 41.0% shared their food with their dog. Forty-eight percent of owners reported having ever disposed of their dog's feces. Twenty-five percent of owners washed their hands after petting their dogs, 6.6% washed their hands before feeding their dog, 77.0% washed their hands before eating their own meals, and 93.1% washed their hands after disposing of their dog's feces.

PFGE results—Eleven (3.6%) isolates were untypeable by use of PFGE because of degradation of genomic DNA during preparation. The use of PFGE identified 205 discrete fecal *E coli* clones, and of these, 169 of 205 (82.4%) clones were isolated from only 1 participant, whereas 36 of 205 (17.6%) clones were shared between multiple (2 to 3) participants. One gel detected 100% PFGE profile similarity between isolates from dog and owner within the same household (**Figure 1**). Thirty-three percent of dogs, 32.7% of owners, and 26.7% of controls shared ≥ 1 fecal *E coli* clone with another participant (range, 1 to 4 shared clones each); there was no difference among the 3 populations ($P = 0.812$). Analysis of dendrograms did not identify clusters of related

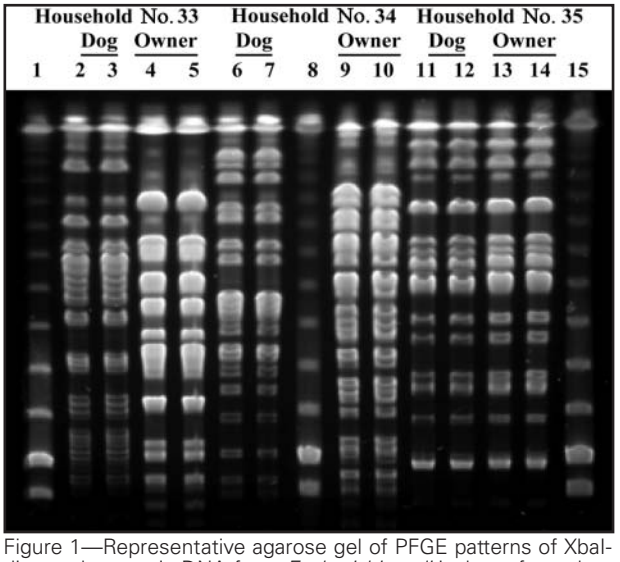

Figure 1—Representative agarose gel of PFGE patterns of XbaI-digested genomic DNA from *Escherichia coli* isolates from dog-owner pairs in 3 households. Lanes 1, 8, and 15 contain low-range pulse field gel markers.[k] Household No. 35 had dog and owner isolates with 100% PFGE profile similarity.

E coli strains that could be traced to a species, population, or occupational exposure.

There were 11,476 potential clone-sharing pairs included in this study; 61 potential within-household clone-sharing pairs and 11,415 potential across-household clone-sharing pairs. Clonal sharing was detected in 36 of 11,476 (0.3%) total potential clone-sharing pairs, 6 of 61 (9.8%) potential within-household clone-sharing pairs, and 30 of 11,415 (0.3%) potential across-household clone-sharing pairs. Within-household sharing was more prevalent than across-household sharing of fecal *E coli* ($P < 0.001$).

Demographic and signalment information was compared with percentage PFGE similarity between isolates from dog-owner pairs, and no significant associations were found with owner gender ($P = 0.253$), dog sex ($P = 0.282$), dog's age category (< 2 years, 2 to 5 years, 6 to 9 years, and ≥ 10 years; $P = 0.563$), breed (purebred or mixed-breed dog; $P = 0.574$), or dog source (breeder, shelter or stray, pet store, or other; $P = 0.123$). Living in a multi-pet versus single pet household was not associated with percentage PFGE profile similarity ($P = 0.226$), nor was total number of pets in the house ($P = 0.069$). Owner affiliation with the University of Tennessee College of Veterinary Medicine was not associated with percentage PFGE profile similarity ($P = 0.829$).

Dog-owner relationship and hand-washing behaviors were compared with PFGE results. Disposal of dog's feces was significantly ($P = 0.013$) associated with percentage PFGE profile similarity; owners who disposed of their dog's feces had *E coli* with a mean percentage PFGE profile similarity with their dog of 81.5%, whereas owners who did not dispose of their dog's feces had *E coli* with a mean percentage PFGE profile similarity with their dog of 85.8% . Times per week owners disposed of feces was associated ($P = 0.027$) with percentage PFGE profile similarity, with owners who disposed of their dog's feces 5 or more times/wk having *E coli* with the lowest percentage PFGE profile similarity with their dog (mean, 77.2%). Hand washing after canine fecal disposal was not associated ($P = 0.516$) with percentage PFGE sharing between dog and owner fecal *E coli* isolates.

Susceptibility results—Susceptibility to all 17 antimicrobial agents was found in 75 of 183 (40.9%) canine isolates, 71 of 183 (38.8%) owner isolates, and 34 of 90 (37.8%) control isolates. All 456 isolates were susceptible to imipenem. Lowest percentage susceptibility in all 3 populations **(Figures 2–4)** was to cephalothin (47.5% susceptibility in dogs, 59.0% in owners, and 60.0% in controls), streptomycin (55.7% in dogs, 63.9% in owners, and 60.0% in controls), and ampicillin (67.2% in dogs, 60.6% in owners, and 50.0% in controls).

No differences were found in susceptibility of *E coli* isolated from dog-owner pairs to any of the 17 antimicrobial agents, whether or not data were collapsed

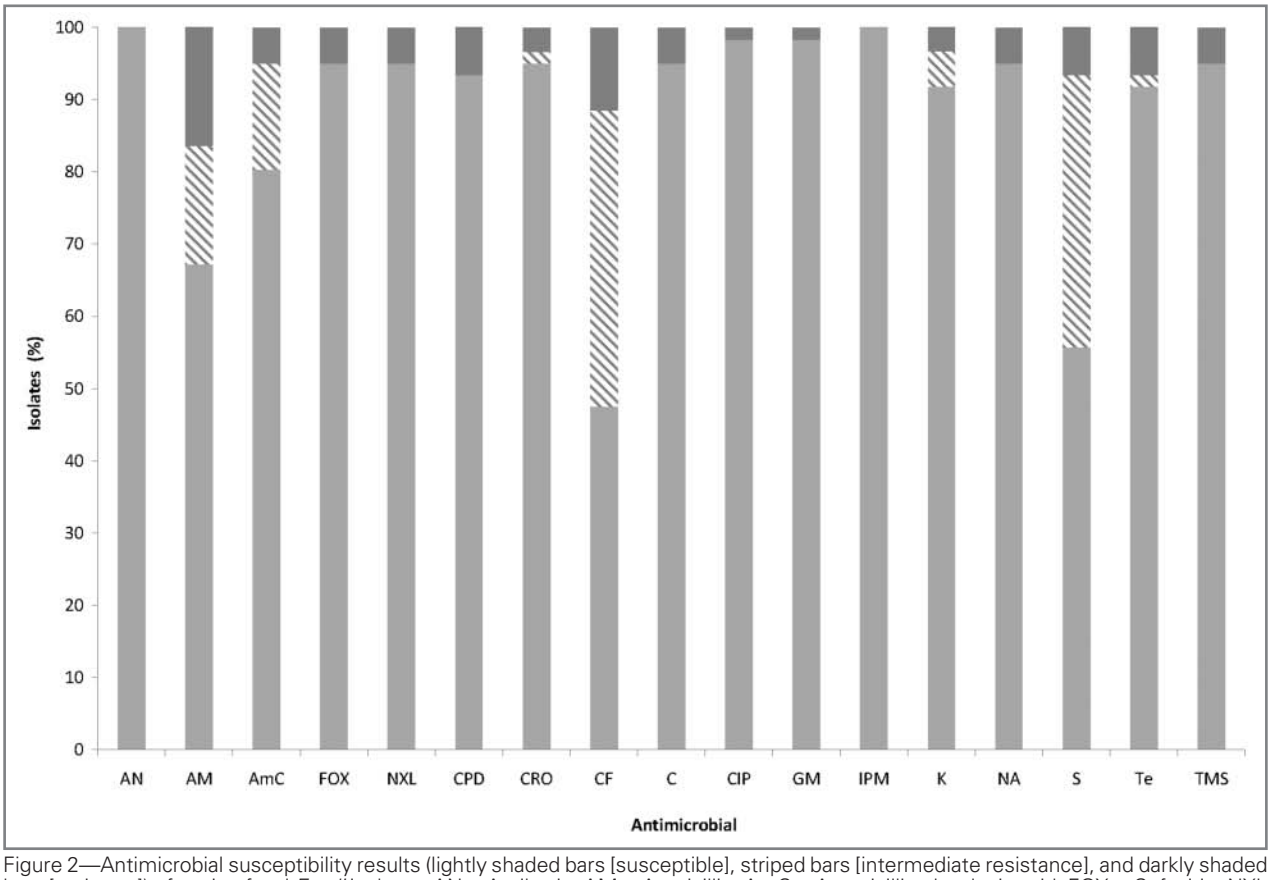

Figure 2—Antimicrobial susceptibility results (lightly shaded bars [susceptible], striped bars [intermediate resistance], and darkly shaded bars [resistant]) of canine fecal *E coli* isolates. AN = Amikacin. AM = Ampicillin. AmC = Amoxicillin-clavulanic acid. FOX = Cefoxitin. NXL = Ceftiofur. CPD = Cefpodoxime. CRO = Ceftriaxone. CF = Cephalothin. C = Chloramphenicol. CIP = Ciprofloxacin. GM = Gentamicin. IPM = Imipenem. K = Kanamycin. NA = Nalidixic acid. S = Streptomycin. Te = Tetracycline. TMS = Trimethoprim-sulfamethoxazole.

by converting intermediate to resistant results. There were no differences in susceptibility results between owner and control isolates, but significant associations were found between canine and control isolates for ampicillin ($P < 0.001$), chloramphenicol ($P = 0.029$), ciprofloxacin ($P = 0.029$), tetracycline ($P = 0.024$), and trimethoprim-sulfamethoxazole ($P < 0.001$), with control isolates more likely resistant than canine isolates to each of these antimicrobial agents. With the Bonferroni adjustment to a threshold value of $P \leq 0.003$, only ampicillin and trimethoprim-sulfamethoxazole remained significant.

Overall, 12.5% of participants had MDR fecal *E coli*. Eight (4.4%) canine isolates were resistant to a range of 3 to 14 antimicrobials (mean, 7.6); 2 dogs had isolates resistant to 7 agents, 1 dog had 3 isolates resistant to 9 agents (each with the same pattern), and 1 dog had an isolate resistant to 14 agents. Five of 8 canine MDR isolates were resistant to 3 or more classes of antimicrobials. Thirty-eight (13.9%) human isolates were MDR, resistant to a range of 3 to 10 antimicrobials (mean, 4.4); 1 owner had 2 isolates each resistant to the same 9 agents, 1 owner had an isolate resistant to 10 agents, and 1 control had 3 isolates resistant to 9 agents (each with the same pattern). Twenty-nine (76.3%) human MDR isolates were resistant to 3 or more classes of antimicrobials. In 2 households (households 2 and 58), MDR *E coli* was isolated from both dog and owner, but isolates within each pair were not closely related on the basis of PFGE analysis. A dendrogram of clonal relatedness of MDR *E coli* isolates from dogs, owners, and controls was created; 3 clusters of MDR isolates with related profiles were identified **(Figure 5)**.

Owner (13.7%) and control (14.4%) isolates were equally likely to be MDR ($P = 0.833$), but control isolates were more likely to be MDR than canine (4.4%) isolates ($P = 0.003$); no significant difference was found between owner and canine isolates. Participants who had received antimicrobial therapy in the month prior to enrollment (but not in the 2 weeks prior to enrollment) were more likely ($P = 0.005$) to carry MDR fecal *E coli* than those who did not receive antimicrobial therapy in this time period.

Owner affiliation with the University of Tennessee College of Veterinary Medicine was associated with susceptibility results to kanamycin ($P = 0.002$), streptomycin ($P = 0.003$), tetracycline ($P = 0.001$), and trimethoprim-sulfamethoxazole ($P = 0.001$). Owners not affiliated with the University of Tennessee College of Veterinary Medicine were more likely to have *E coli* resistant to each of these antimicrobials than owners affiliated with the University of Tennessee College of Veterinary Medicine.

Hand washing after petting their dogs was associated ($P = 0.003$) with susceptibility results of owners' fecal *E coli*. Fifty percent of fecal isolates from owners who did not wash their hands after petting their dog were ampicillin resistant, whereas 4.4% of fecal isolates from owners who did wash their hands were ampicillin resistant.

Hand washing before meals was associated with higher susceptibility of owners' fecal *E coli* to chloram-

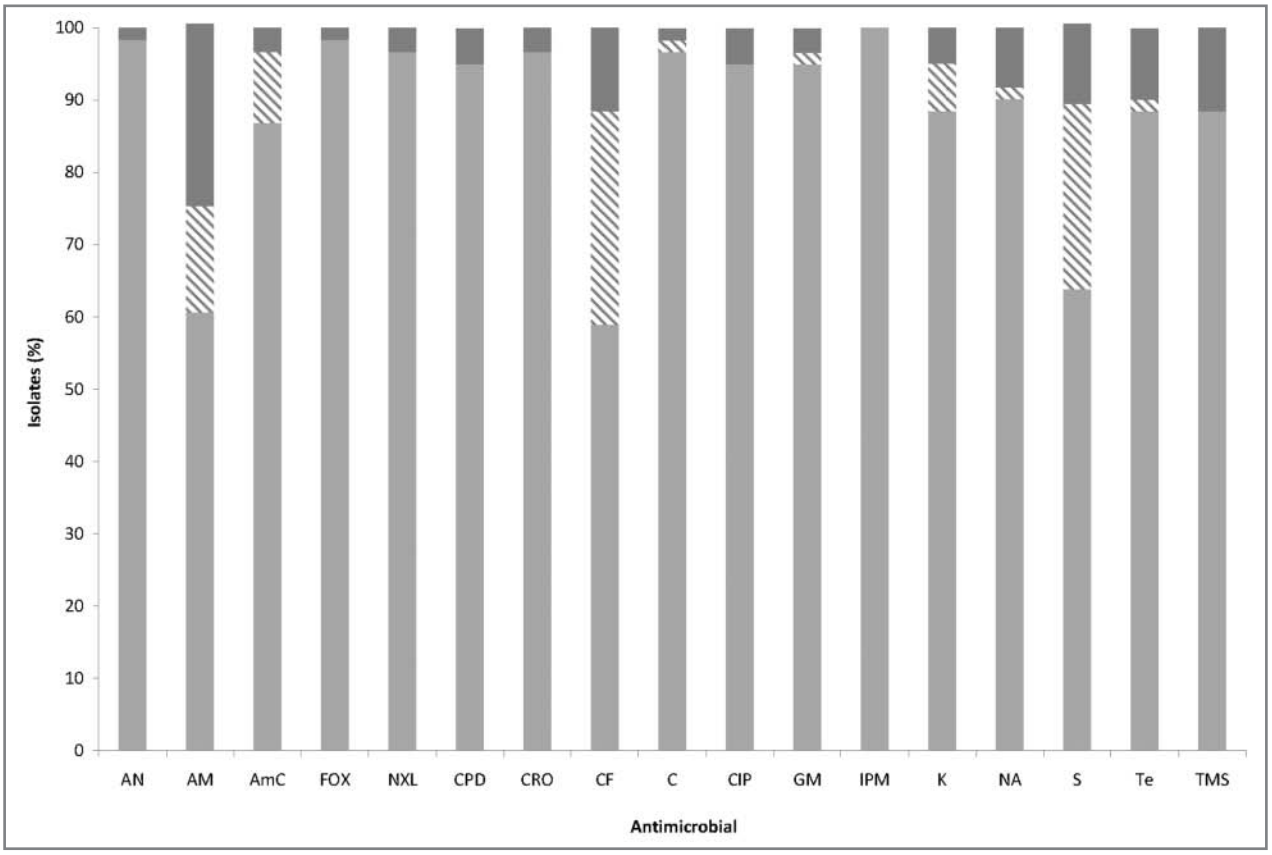

Figure 3—Antimicrobial susceptibility results of owner fecal *E coli* isolates. *See* Figure 2 for key.

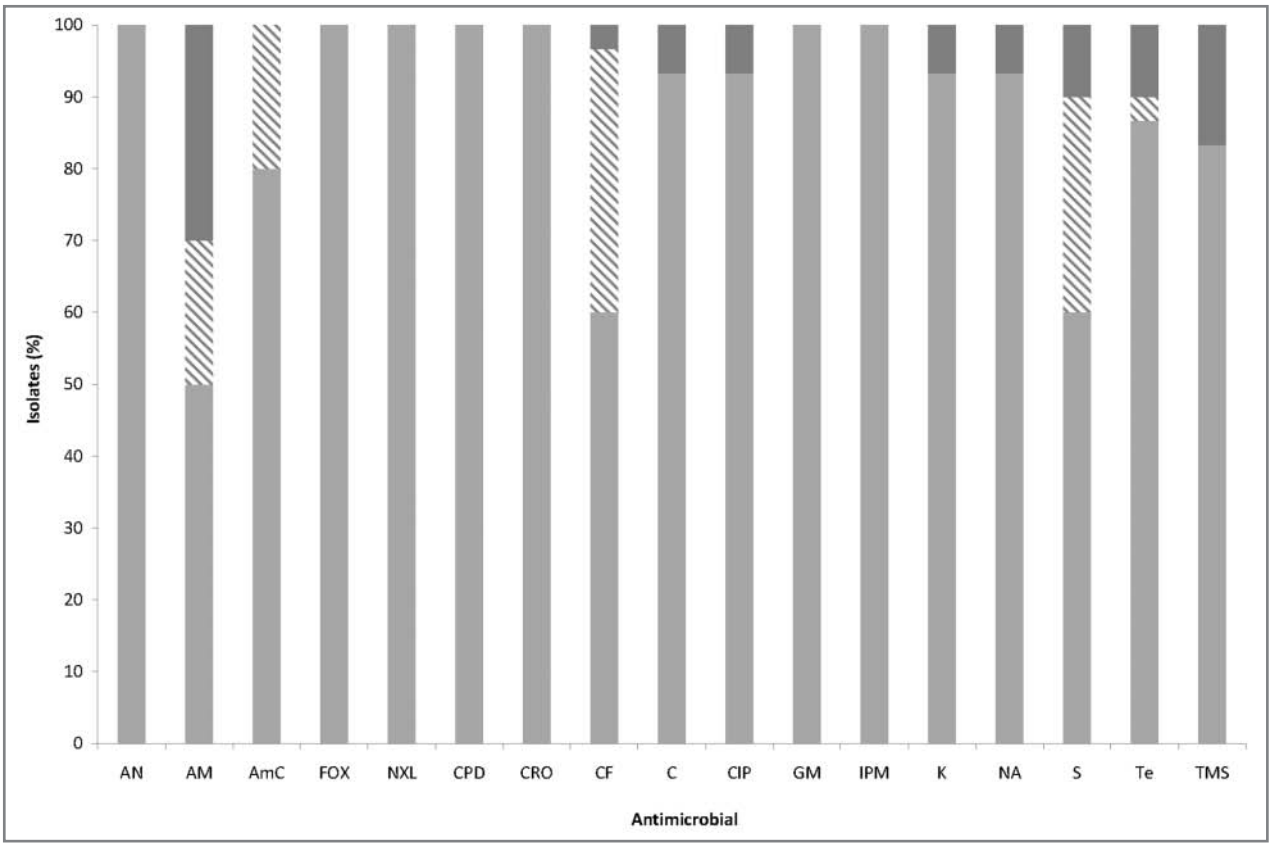

Figure 4—Antimicrobial susceptibility results of fecal *E coli* isolates from control humans. *See* Figure 2 for key.

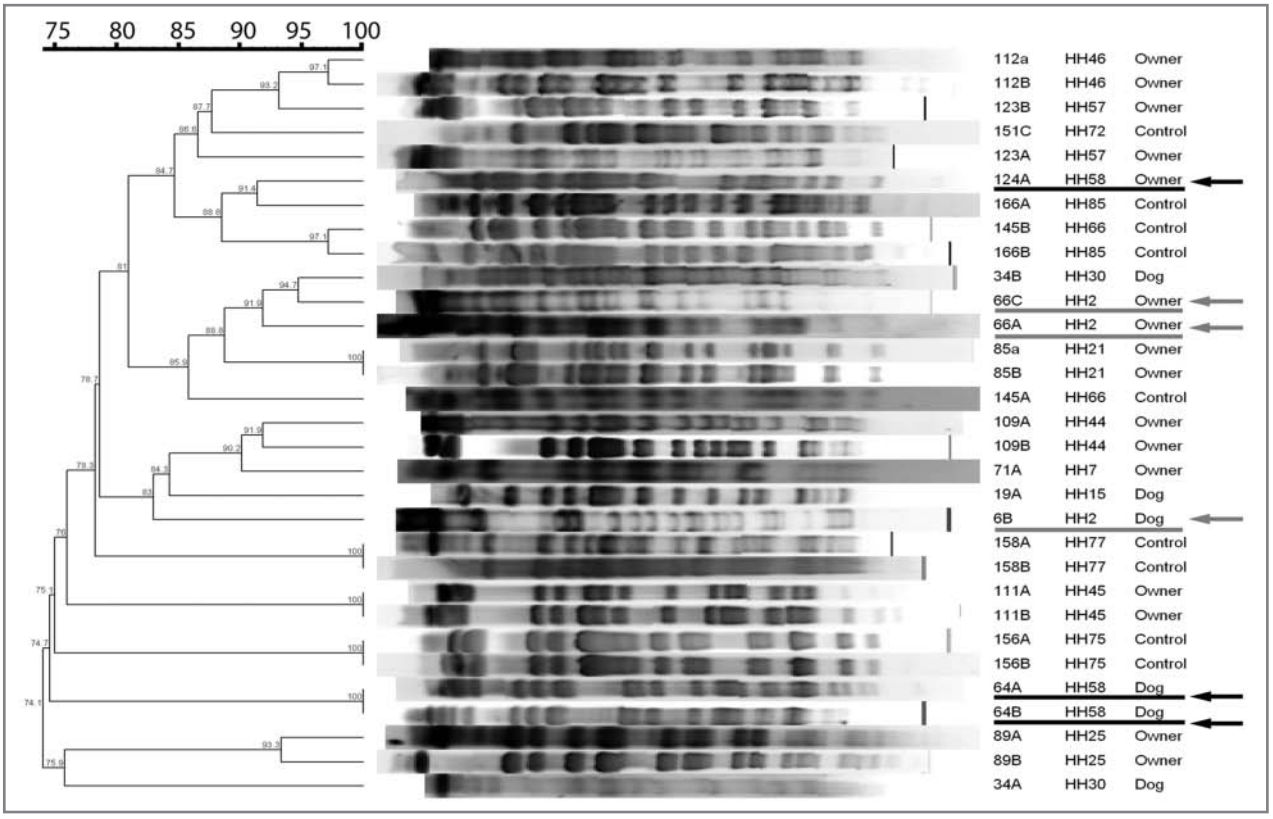

Figure 5—Dendrogram of MDR isolates from dogs, owners, and control humans. The scale on the left side of the figure indicates the degree of relatedness. From 2 households (HH2 [gray arrows] and HH58 [black arrows]), MDR *E coli* was isolated from both dog and owner. Three clusters of related MDR profiles were identified. HH = Household.

phenicol ($P < 0.001$), ciprofloxacin ($P < 0.001$), and nalidixic acid ($P < 0.001$). Fecal isolates from owners who did not wash their hands before their own meals were more likely resistant to chloramphenicol (7.1% vs 0%), ciprofloxacin (14.3% vs 0.7%), and nalidixic acid (14.3% vs 2.8%) than fecal isolates from owners who did wash their hands before meals. There were no significant associations identified between owners' hand washing prior to feeding dogs and susceptibility results in canine fecal *E coli*.

Dogs drinking water out of the toilet was associated with susceptibility results in canine fecal *E coli* to ciprofloxacin ($P < 0.001$) and nalidixic acid ($P = 0.002$). Dogs that drank water out of the toilet were more likely to have fecal *E coli* resistant to ciprofloxacin (12.5% vs 0%) and nalidixic acid (12.5% vs 1.3%) than dogs that did not drink water out of the toilet.

Susceptibility patterns were not consistent within a single clone of *E coli*. Nine of 36 (25.0%) isolates of shared *E coli* clones had identical susceptibility patterns. No associations were found between antimicrobial susceptibility and percentage PFGE similarity.

Discussion

Definitions of clonal sharing vary, including 90%, 94%, or 100% PFGE profile similarity cutoffs or comparison of bands in common among strains.[6,8,13,14] The 94% cutoff was chosen to be most consistent with current literature in this field[6,8]; however, by use of a 90% cutoff as used by Cooke et al,[13] within-household prevalence in our study would have increased from 9.8% to 21.3%, indicating that choice of definition is an important factor that could sway the outcome of a study.

The PFGE profiles of 205 *E coli* isolates varied widely, with 82.4% identified from a single host. These findings were in agreement with those of a previous study,[8] which also used a definition of ≥ 94% PFGE profile similarity to signify clonal sharing, that enrolled 63 households with 152 humans and 76 pets and found 335 unique fecal *E coli* clones with 73% from a single host. These data suggest there is a great variety of *E coli* strains shed in the feces of healthy humans and dogs into the environment.

The present study found that dogs (32.7%) and humans (owners, 32.7%; controls, 26.7%) were equally likely to share *E coli* clones with other participants. Similarly, the previous study[8] found 52% of participants shared a clone with another participant, with pets (52%) sharing clones no more frequently than humans (53%).

Prevalence data revealed that within-household sharing occurred more often (9.8%) than across-household sharing (0.3%). This finding was similar to a previous finding of 27% within-household sharing and 0.8% across-household sharing.[8] A potential explanation for the previous study[8] identifying a higher prevalence of within-household sharing is because that study included up to 20 isolates/participant and multiple humans and pets per household.

Although a large variety of *E coli* was identified, some genetically similar strains were shared by individuals with no known contact. Although occupations may provide common exposure to environmental reservoirs of *E coli*, no single clone was seen in a large proportion of participants as would be expected from a single point source. Other common environmental reservoirs of *E coli*, such as water and food supplies, could also explain across-household sharing.

A study[15] of the human-animal bond identified indicators of bond strength, including willingness to spend money on a dog, considering their dog a child, and missing their dog when away from home, and found that female owners were more bonded with their dogs than were male owners; however, the present study found no difference in clonal sharing or antimicrobial susceptibility with regard to owner gender or dog sex. Owners who purchased their dogs shared stronger bonds with their dogs than owners who acquired their dogs at no cost from family or friends,[15] but no differences in clonal sharing between dog and owner were found in the present study with regard to source of dog acquisition or purebred status. The high percentage of multi-pet households (82.0%) was expected in this study because of recruitment from the University of Tennessee College of Veterinary Medicine and was greater than that in the general public (59%),[15] but neither number of pets in household nor affiliation with the University of Tennessee College of Veterinary Medicine was associated with clonal sharing between enrolled dog-owner pairs. In contrast, a previous study[8] found that within-household sharing of *E coli* increased linearly with the total number of humans and pets in the household.

The association between disposing of canine feces and decreased clonal relatedness may relate to owners who frequently handle their dog's feces having better hygiene and washing their hands more consistently, but this association was not proven statistically. Despite these results, it would be irresponsible to suggest that handling canine feces is protective against cross-species bacterial sharing, and good hygiene with hand washing after handling feces is recommended.

In both canine and human participants, susceptibility of fecal *E coli* was lowest to ampicillin, cephalothin, and streptomycin. Lower susceptibility to β-lactam antimicrobials may be attributable to presence and plasmid-mediated spread of genes for β-lactamases as well as selection pressure. Two human participants and 1 dog received β-lactam therapy in the month prior to enrollment, but 26 courses of β-lactam therapy were received by participants in the year prior to enrollment, and resistant *E coli* from these participants may have contaminated local environments, contributing to overall β-lactam resistance.

Although streptomycin is no longer widely used in clinical medicine, it is a naturally produced antimicrobial and natural exposure may maintain selection pressure and resistance. Although molecular testing for resistance genes was not performed in this study, low streptomycin susceptibility may also be attributable to presence and horizontal spread of resistance genes on class 1 integrons and plasmids.[16,17]

Failure to find significant differences in antimicrobial susceptibility results of *E coli* isolated from dogs and their owners could be attributable to low study power. Alternatively, within-household transmission of plasmids or integrons carrying genes for antimicrobial resistance, either directly or through environmental

reservoirs, may have occurred, leading to similar susceptibility profiles between dogs and owners. Skurnik et al[18] found that fecal isolates from animals free of human exposure had no antimicrobial resistance, and there was a steady increase in resistance as exposure to humans increased. Class 1 integrons were not seen in isolates from wild animals but were seen in isolates from farm animals (7% prevalence), pet dogs (16% prevalence), and humans (16% prevalence).[18] Those results suggest that transmission of mobile genetic units would be more likely in the direction of owners to dogs than dogs to owners, but further research in this area is warranted.

Lack of significant differences between susceptibility results of *E coli* isolates from owners and controls could also have resulted from low power but suggested that ownership of a healthy dog did not increase antimicrobial resistance of a human's fecal *E coli*. This finding has important public health implications, especially as concerns arise regarding antimicrobial-resistant bacteria being isolated from pets.[3,6,16,19,20] Dogs provide psychologic and physiologic support to their owners, and these results support claims that risk of zoonotic transmission of resistant bacteria, specifically nonpathogenic *E coli*, is minimal.[21] Regardless, proper hygiene recommendations such as hand washing should be followed by all pet owners, especially in households with children and elderly or immunosuppressed adults.

Increased resistance to ampicillin and trimethoprim-sulfamethoxazole in control isolates over canine isolates may have resulted from selection pressure, different exposure to resistant *E coli* in the environment, or sampling error. Selection pressure may have been a factor in 1 control who had received an unspecified sulfonamide antimicrobial in the month prior to enrollment, whereas no dogs had received sulfonamide therapy prior to the study. No controls or dogs had received ampicillin or amoxicillin in the month prior to enrollment.

Increased isolation of MDR *E coli* from human participants, compared with dogs, may have resulted from occupational exposure to MDR strains, with 25.2% of participants working in veterinary clinics and 21.9% of participants working in human hospitals; sampling the general public may not have yielded the same results. Alternatively, humans may have carried more MDR *E coli* because of differences in exposure to antimicrobials and resistant *E coli* in the environment. Our results concur with the findings of Sannes et al[5] and Skurnik et al[18] that MDR *E coli* is more likely to be transferred from owner to dog than from dog to owner.

Several isolates from dogs and humans in the present study were resistant to a high number of antimicrobials from multiple classes. The study of Sannes et al[5] found similar percentages of MDR isolates from canine feces (2/45 [4.4%]) and human feces (6/76 [7.9%]), but their isolates were resistant to either a combination of ampicillin, amoxicillin-clavulanate, and cefoxitin or a combination of ampicillin, sulfisoxazole, trimethoprim, and trimethoprim-sulfamethoxazole.[5] In the 2 households in which MDR *E coli* was isolated from both dog and owner, the high degree of resistance may be explained by selection pressure from recent antimicrobial therapy followed by sharing of plasmids or class 1 integrons through direct contact or environmental reservoirs within the households.

Selection pressure of prescription medications was supported by the association found between receiving antimicrobials within 1 month of enrollment and isolation of fecal MDR *E coli*. Antimicrobial use is considered by some to be the most important factor in the emergence, selection, and dissemination of antimicrobial-resistant bacteria.[22] Judicious prescribing of antimicrobials is recommended for veterinarians and physicians to minimize selection pressure and future development of resistant organisms.

Differences in susceptibility associated with affiliation with the University of Tennessee College of Veterinary Medicine may be attributable to occupational exposure to antimicrobial-resistant *E coli*. Many non–University of Tennessee College of Veterinary Medicine participants were affiliated with human hospitals and may have had occupational exposure to resistant *E coli*. Differences in practices regarding antimicrobial prescriptions in veterinary and human hospitals may also influence the flora of veterinary versus human hospital workers.

Hand washing is an important way to minimize spread of bacteria. In theory, transmission of resistant *E coli* or plasmids carrying resistant genes could occur in either direction if owners do not wash their hands before or after petting their dogs. Ampicillin-resistant *E coli* was isolated more often from the feces of owners who did not wash their hands after petting their dogs than from those owners who did wash, suggesting potential transmission by this route. Regrettably, frequency of hand washing prior to petting dogs was not included in the questionnaire. This study found that hand washing prior to owners' meals was associated with a lower percentage of *E coli* isolates resistant to chloramphenicol, ciprofloxacin, and nalidixic acid than from owners who did not wash their hands before meals. These findings support recommendations for hand washing by owners after petting dogs and prior to meals. Although advisable, no specific evidence was found in this study to support benefits of hand washing prior to petting dogs and dog meal preparation.

Our data revealed that dogs reported to drink water out of the toilet were more likely to carry fluoroquinolone-resistant *E coli* than dogs that did not drink from the toilet ($P < 0.001$ for ciprofloxacin). This suggests that toilets may be a reservoir for fluoroquinolone-resistant *E coli*. Until further studies determining the magnitude of this potential risk have been performed, we recommend that owners discourage dogs from drinking water from toilets.

This study would have been strengthened by a longitudinal study design and increased number of isolates per participant because *E coli* clones can be detected inconsistently in a particular individual.[6] Including multiple humans of all ages and pets of all species from each household may have reduced type II error and increased power for detecting differences in susceptibility and cross-species clonal sharing.[6,8] Performing PFGE on all 3 isolates for each participant would have increased sensitivity for detecting sharing. Disk diffusion

susceptibility testing could have been complimented by molecular testing for presence of resistance genes.

Sharing of clonal *E coli* between dog-owner pairs occurred with 9.8% prevalence, with no differences found in susceptibility patterns. No specific behaviors were identified that were associated with increased sharing of fecal *E coli* clones between dogs and their owners, but lack of hand washing after petting dogs and prior to owners' meals was associated with isolation of resistant *E coli*. Direct contact between dogs and owners may be responsible for transmission of bacteria and resistance genes within households, and good hygiene, including frequent hand washing, is recommended. Sharing of fecal *E coli* also occurred across households, suggesting environmental reservoirs of *E coli* may be important. Public health concerns regarding cross-species sharing of *E coli* are warranted, and efforts such as proper hygiene measures to minimize transmission should be taken.

a. BBL CultureSwab Plus, Becton Dickinson and Co, Sparks, Md.
b. MUG Fluorescence Crystals, Hach Chemical Co, Loveland, Colo.
c. EC Medium with MUG, Becton Dickinson and Co, Sparks, Md.
d. API 20E test kits, bioMérieux Inc, Durham, NC.
e. Proteinase K, Qiagen Inc, Valencia, Calif.
f. 10% Sarkosyl Solution, Teknova, Hollister, Calif.
g. Buffer D, Promega, Madison, Wis.
h. Bovine serum albumin, Promega, Madison, Wis.
i. XBA I, Promega, Madison, Wis.
j. CHEF-Mapper, BioRad Laboratories, Richmond, Calif.
k. Low Range PFG Marker, New England BioLabs Inc, Ipswich, Mass.
l. FPQuest Software, version 4.5, BioRad Laboratories, Richmond, Calif.
m. SPSS, version 15.0, SPSS Inc, Chicago, Ill.

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