

Antimicrobial Resistance Profiles and Clonal Relatedness of Canine and Feline *Escherichia coli* Pathogens Expressing Multidrug Resistance in the United States

B.W. Shaheen, D.M. Boothe, O.A. Oyarzabal, and T. Smaha

Background: Antimicrobial resistance is increasing among *Escherichia coli* isolates associated with spontaneous infection in dogs and cats.

Objectives: To describe *E. coli* resistance phenotypes and clonal relatedness and their regional prevalence.

Animals: Isolates of *E. coli* (n = 376) collected from dogs and cats in the United States between May and September 2005.

Methods: Isolates submitted from the South, West, Northeast, and Midwest regions of the United States were prospectively studied. Phenotype was based on E-test susceptibility to 7 antimicrobials. Isolates were classified as no (NDR), single (SDR), or multidrug resistance (MDR). Clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE).

Results: One hundred and ninety-three (51%) isolates expressed resistance to at least 1 drug, yielding 42 phenotypes. SDR isolates (n = 84; 44%, 8 phenotypes), expressed resistance most commonly to amoxicillin (30%, n = 25) and least commonly to cefpodoxime (1%, n = 1). MDR isolates (n = 109; 56%, 31 phenotypes) were resistant to amoxicillin (96%, n = 105), amoxicillin-clavulanate (85%, n = 93), and enrofloxacin (64%, n = 70); 18% (n = 20) were resistant to all drugs tested. The frequency of MDR did not differ regionally (P = .066). MDR minimum inhibitory concentrations (MICs) were 6-fold higher than SDR MICs (P < .0001). Dendrograms of 91 isolates representing 25 phenotypes revealed 62 different PFGE profiles.

Conclusions and Clinical Importance: *E. coli* strains spontaneously infecting dogs and cats are genetically and phenotypically diverse. Given the current prevalence of MDR among clinical isolates of *E. coli* in United States, implementation of a robust surveillance program is warranted.

Keywords: Antimicrobial resistance; Pulsed-field gel electrophoresis; Susceptibility testing

The emergence of antibiotic resistance has been documented in *Escherichia coli* isolates from human, animal, and environmental sources.¹ Multidrug resistant (MDR) *E. coli* is an emerging health concern.² Several studies have demonstrated an increase in MDR *E. coli* associated with infections in dogs and cats throughout both the United States and Europe.^{2–5} Nosocomial infections associated with MDR *E. coli* have been reported in dogs in intensive care units. The extensive use of broadspectrum antimicrobials is a likely contributing factor for MDR *E. coli*.⁶

In addition to the impact on animal health, emergent MDR *E. coli* might have important public health consequences if isolates are transmitted between humans and their pets.^{7,8} The phylogenetic and pathotypic similarities between *E. coli* isolates from urinary tract infections in dogs and extraintestinal pathogenic *E. coli* (ExPEC) isolates from humans are an example of a cause for concern.^{9–11} Over 15% of fecal specimens from dogs were found to contain *E. coli* strains closely related to virulent ExPEC clones isolated from humans.¹²

From the Department of Anatomy, Physiology, and Pharmacology, Auburn University, Auburn, AL (Shaheen, Boothe, Smaha); and the Department of Biological Sciences, Alabama State University, Montgomery, AL (Oyarzabal). This research work was performed at the Clinical Pharmacology Laboratory, College of Veterinary Medicine, Auburn University. A portion of the work was presented as a poster presentation at the 2008 ACVIM Forum, San Antonio, TX.

Corresponding author: D. M. Boothe, DVM, MS, PhD, DACVIM, DACVCP, Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, 109 Greene Hall, Auburn, AL 36849; e-mail: boothdm@auburn.edu.

Submitted May 29, 2009; Revised October 17, 2009; Accepted November 27, 2009.

Copyright © 2010 by the American College of Veterinary Internal Medicine

10.1111/j.1939-1676.2009.0468.x

Abbreviations:

A	amoxicillin
AX	amoxicillin-clavulanic acid
D	doxycycline
E	enrofloxacin
ExPEC	extraintestinal pathogenic <i>E. coli</i>
G	gentamicin
MDR	multidrug resistance
MIC	minimum inhibitory concentration
NDR	no drug resistance
P	cefpodoxime
PFGE	pulsed-field gel electrophoresis
SDR	single drug resistance
T	trimethoprim-sulfamethoxazole
TIFF	tagged image file format

Molecular characterization of resistant isolates utilizing fingerprinting techniques such as pulsed-field gel electrophoresis (PFGE), repetitive extragenic palindromic polymerase chain reaction analysis, and genetic sequencing are important tools to describe the spread of bacterial clonal units. Among these, PFGE is particularly useful to demonstrate close relationships among strains, including those manifesting MDR.

Understanding the patterns of antimicrobial resistance in canine and feline *E. coli* isolates is important not only from a veterinary medical prospective, but also from a global public health prospective. MDR bacteria are detected with increasing frequency in populations exposed to antimicrobial drugs.³ Therefore, it is increasingly important for veterinarians to understand the impact of antimicrobial drug use and the potential for emergence of antimicrobial resistance patterns. In the present study, we characterize the specific phenotypes of resistant *E. coli*

isolates, including a description of the resistance among antimicrobial drugs, the clonal relatedness of the isolates, and the level of resistance. The resistance described herein may impact empirical selection of antimicrobial drugs when treating canine or feline *E. coli* infections.

Materials and Methods

Sample Collection and Identification

All *E. coli* isolates (n = 376) obtained from each participating laboratory were originally submitted to the participating laboratory by practicing veterinarians. As such, the site of collection from various tissues (eg, urine, skin, ear, lung, and others) was determined by the presenting population and all isolates were assumed to be pathogenic, that is, associated with a clinical infection. To minimize bias in sample submission from the laboratories, each laboratory was directed to submit all *E. coli* isolates from dogs or cats received between May and September 2005. Of these isolates, all samples received from each participating laboratory except those collected from feces or anal sacs were studied. Samples were subjected to each laboratory's standard procedures for culture and susceptibility testing. After sample processing, pure cultures of each isolate were established on a trypticase agar slant and shipped overnight at room temperature by the participating laboratory to the Clinical Pharmacology Laboratory at Auburn University. Contributing laboratories included 4 commercial and 5 academic (university-based) veterinary microbiologic laboratories. The commercial laboratories were located in California, Indiana, Massachusetts, and Wisconsin. The academic laboratories were located in Alabama, Mississippi, North Carolina, Kansas, and Washington. Based on the state of origin of the submitting veterinarian, samples were divided into 4 geographical regions: South, including the states of Alabama, Mississippi, and North Carolina; West, including the states of Washington and California; the Midwest, including Indiana, Kansas, and Wisconsin; and Northeast, represented by the state of Massachusetts. On arrival at the Clinical Pharmacology laboratory, isolate identity was further confirmed after incubation on MacConkey agar plates and Kovacs testing.^b All submitted isolates, except those collected from feces or anal sacs, were studied.

Susceptibility Testing

On receipt, isolates were streaked on trypticase agar and incubated at 37°C for 24 hours. Samples were then adjusted to 0.5 McFarland standard turbidity ($\sim 10^8$ CFU), and a sterile swab was used to inoculate two 150 mm Mueller Hinton plates (MH). Susceptibility to 7 drugs, representing 5 classes of antimicrobials, was determined by the E-test^c according to the manufacturer's instructions. The minimum inhibitory concentration (MIC) results were interpreted according to criteria established by the Clinical and Laboratory Standards Institute (CLSI).¹³ Drugs tested included 3 β -lactams (amoxicillin, amoxicillin-clavulanic acid [penicillins], and cefpodoxime [a 3rd generation cephalosporin]), a tetracycline (doxycycline), a fluoroquinolone (enrofloxacin), an aminoglycoside (gentamicin), and a "potentiated" sulfonamide (trimethoprim-sulfamethoxazole). Drugs were selected based on different mechanisms of antimicrobial action and their historical use by canine and feline veterinary practitioners. After incubation of the MH plates at 35°C for 20–24 hours, the MIC of each drug for each isolate was recorded, and the isolate was designated as resistant (R) or susceptible (S) to each the 7 drugs. The CLSI intermediate designation (I) was treated as R.¹⁴ For control purposes, *E. coli* ATCC 25922^d (cefpodoxime MICs range = 0.25–1 μ g/mL) and *Streptococcus*

pneumoniae ATCC 49619^e (amoxicillin MICs range = 0.032–0.125 μ g/mL) were used.

Characterization of Antimicrobial Resistance Phenotypes

Antimicrobial resistance phenotypes were generated for each isolate and categorized as no drug resistance (NDR), resistant to a single drug or drug class (SDR) (amoxicillin, amoxicillin-clavulanic acid, and cefpodoxime considered a single drug class) or resistant to 2 or more classes of antibacterial agents (MDR).¹⁵ Those MDR isolates that were resistant to all 7 antimicrobials tested were designated as "extreme" drug resistance, or XDR. The isolates' phenotypic expression in each region or each tissue site was determined. The magnitude level of resistance, based on mean MIC, was compared among isolates expressing SDR and MDR phenotypes. Isolates with an MIC greater than the maximum concentration on the E-test (exceeding the CLSI resistant MIC breakpoint by at least 8-fold) were considered to express high-level resistance.

Statistical Analysis

Associations between the various categories, including geographical and tissue sites and the type of resistance (NDR, SDR, or MDR [including XDR]), were assessed by the χ^2 test by MINITAB 15 statistical package.^e Statistical significance was considered at a *P* value of $\leq .05$. Additionally, MICs were compared among the different categories of resistance for each drug using a Student's *t*-test after converting MIC to geometric mean.

Clonal Relatedness of Canine and Feline *E. coli* Isolates

Any phenotype in each region represented by at least 2 isolates was subjected to genetic fingerprinting by PFGE according to protocols developed by the Centers for Disease Control and Prevention (Atlanta, GA).¹⁶ Briefly, the genomic DNA of each isolate was embedded in agarose plugs and the plugs were transferred to 50 mL tubes^f containing 5 mL of cell lysis buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% sarcosine, and 0.1 mg of proteinase K/mL). Samples (n=91 isolates) were lysed for 120 minute at 54°C in an orbital shaker water bath with constant agitation (150–200 rpm). A total of 6 washes (twice with sterile ultrapure water and 4 times with 0.01 M Tris-EDTA buffer, pH 8.0) were used to remove excess reagents and cell debris from the lysed samples. Samples were treated with the restriction endonuclease *Xba*I^g (40–50 U of enzyme at 37°C for 2 hours) and PFGE was performed with a contour-clamped homogeneous electric field machine.^h Running parameters for DNA electrophoresis were 18 hours at a constant voltage of 200 V (6 V/cm), with a pulse time of 5.0–40.0 seconds, an electric field angle of 120°, and a temperature of 14°C. Completed gels were stained with ethidium bromide.

Computer Analysis of PFGE Patterns

Gel images were captured on a Gel Doc imaging system.ⁱ Images were converted to tagged image file format (TIFF) files using the automated GeneSnap^j and analyzed with BioNumerics software.^k The TIFF images were normalized using 3 standards per gel (*Salmonella* serotype Braenderup H9812), which were loaded on lanes 1, 7, and 15.¹⁷ Matching and dendrogram analysis of the PFGE patterns were accomplished by unweighted pair group method with arithmetic mean analysis and the Dice correlation coefficient. Samples with DNA relatedness of 90% or more were considered identical.

Results

Antimicrobial Resistance Phenotypes

Of the 376 isolates studied, 301 samples were collected from dogs and 75 from cats. The most common samples were urine (n = 174), skin (n = 27), lung (n = 22), ear (n = 27), soft tissue (n = 15), and others (n = 111). Resistance to at least 1 drug or drug class was expressed by 193 (51.3%) isolates. Resistant isolates were represented by 43 different phenotypes: 8 phenotypes for SDR (n = 84 isolates) (Fig 1A) and 35 phenotypes for MDR isolates (n = 109 isolates) (Fig 1B). Within MDR, most phenotypes were represented by at least 2 isolates (total n = 93) with 16 phenotypes represented by only 1 isolate (the latter are not indicated in Fig 1B). Among the SDR phenotypes, the most commonly represented class of drugs to which resistance was expressed was the β-lactams (82%, n = 69/84). This included amoxicillin (30%, n = 25/84) and amoxicillin-clauvalanic acid (29%, n = 24/84) (Fig 1A). Other strains were resistant to combinations of amoxicillin, amoxicillin-clauvalanic acid, and cefpodoxime (24%, n = 20/84). Less than 20% (n = 14/84) of the SDR

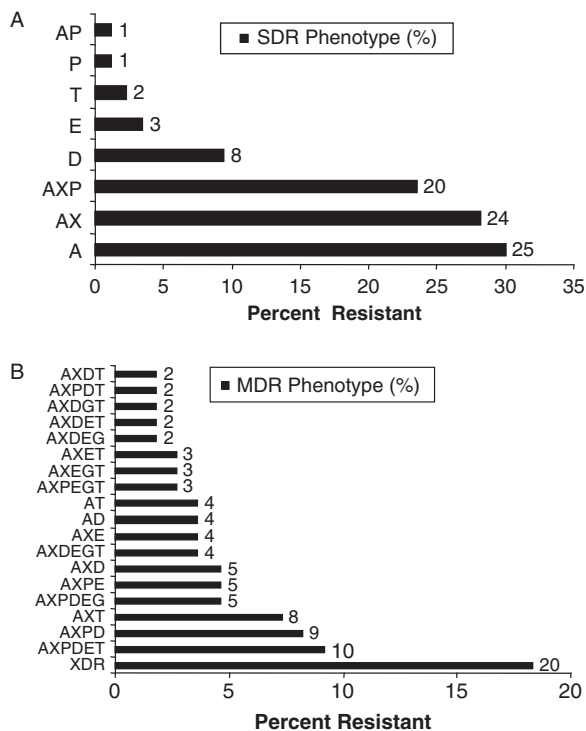


Fig 1. The number and proportion (%) of each SDR (A) and MDR (B) phenotype(s) expressed in 376 isolates of *Escherichia coli* causing spontaneous disease in dogs and cats throughout the United States. Numbers of isolates are indicated above each bar. The resistant isolates were represented by 43 different phenotypes including 8 phenotypes for SDR isolates (n = 84) and 35 phenotypes for MDR isolates (n = 109). MDR phenotypes with 1 isolate (n = 16) and NDR phenotypes (n = 183) are not indicated in (B). SDR, single drug resistance; MDR, multiple drug resistance; NDR, no drug resistance; XDR, resistance to all 7 antimicrobials; A, amoxicillin; AX, amoxicillin-clauvalanic acid; P, cefpodoxime; D, doxycycline; E, enrofloxacin; G, gentamicin; T, trimethoprim-sulfamethoxazole.

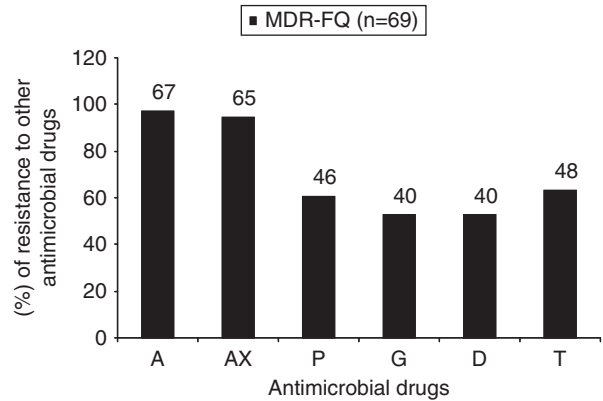


Fig 2. Frequency of resistance to specific antibiotics in isolates with MDR phenotypes that are resistant to enrofloxacin. Numbers of isolates are indicated above each bar. MDR, multiple drug resistance; FQ, flouroquinolone; A, amoxicillin; AX, amoxicillin-clauvalanic acid; P, cefpodoxime; D, doxycycline; E, enrofloxacin; G, gentamicin; T, trimethoprim-sulfamethoxazole.

isolates were resistant to cefpodoxime, doxycycline, enrofloxacin, or trimethoprim-sulfamethoxazole alone (Fig 1A).

For MDR (n = 109), the most common phenotype was XDR, with 18% (n = 20), expressing resistance to all 7 antimicrobials, while the resistance percentages to 5 and 6 drugs were 17% (n = 16) and 21% (n = 23), respectively (Fig 1B). The remaining MDR isolates were represented by 31 different phenotypes. Amoxicillin was associated with 96% (n = 105/109) of the MDR phenotypes, followed by amoxicillin-clauvalanic acid (85%; n = 93/109), and enrofloxacin (64%; n = 70/109). Comparison for statistical significance for this association was not performed because of insufficient observations and limited sample size. The proportion of each drug that was co-resistant with enrofloxacin was determined. For enrofloxacin, only 3 of 84 (4%) isolates were SDR. β-lactams were the most common drug accompanying enrofloxacin resistance in isolates expressing MDR (Fig 2). The drugs with which enrofloxacin resistance was most commonly associated with were amoxicillin (97%), amoxicillin-clauvalanic acid (94%), and cefpodoxime (67%). The enrofloxacin-MDR phenotype also was present in the majority of isolates expressing resistance to trimethoprim-sulfamethoxazole (63%), cefpodoxime (60%), gentamicin (53%), and doxycycline (53%) observed.

The majority of isolates were submitted by the South region (n = 133), while the West submitted 79 isolates (Table 1). In the South, the largest proportion of resistant isolates were submitted by Auburn University 31% (n=41), followed by Mississippi 20% (n = 26), and North Carolina 20% (n=27) representing the academic-based laboratories. Isolates from the South region had a higher proportion of resistance (62%), while the West region had the lowest proportion of resistance compared with other regions (38%) (P value < .001). Among different phenotypes found in the South region, the MDR phenotype was represented by more XDR than non-XDR phenotypes (28%, n = 16/57) (Table 1).

Table 1. Distribution of resistant phenotypes and their geographical locations.

Regions (Total Number)	Number of Resistant Phenotypes (%) ^a	Antimicrobial Phenotype(s) ^b
Midwest (107)	53 (50)	A (10); AD, AXDEGT, AXET, AXP, AXPD, AXPDET, E (2); ADG, AT, AXD, AXDE, AXDET, AXE, AXEGT, AXPDEG, AXPDT, AXPEGT, AXPET, AXPGT, AXT, DE, DEG, P, T, XDR (1); AX (6); D (5)
Northeast (57)	27 (47)	A, AX (4); ADEGT, AXDGT, AXDT, AXE, AXPDEG, AXT, E (1); AXDEGT, AXPD (2); AXP (5); XDR (3)
South (133)	83 (62)	A, AXPE (5); AD, ADET, ADGT, AP, AXD, AXDET, AXDGT, AXDT, AXET, AXPDGT, AXPDT, AXPEG, AXPEGT, AXPT, AXT, T (1); AT, AXE, AXDEG, AXEGT, AXEGT, AXP, AXPDEG, D (2); AX, AXP (9); AXPDET (8); XDR (16)
West (79)	30 (38)	A (6); AD, AT, AXPDE, AXPDEG, AXPEGT, AXT, D, DT, EGT (1); AX (5); AXD, AXP (3); AXP (4)

^aNumber in parentheses is the percent of resistant isolates collected from that region.

^bNumber in parentheses is the total number of phenotypes collected from that region.

A, amoxicillin; X, amoxicillin-clavulanic acid; P, cefpodoxime; D, doxycycline; E, enrofloxacin; G, gentamicin; T, trimethoprim-sulfamethoxazole; XDR, resistance to all 7 antimicrobials.

Comparisons of Antimicrobial Resistance Profiles among Isolates from Different Geographical Regions and Tissues

The proportion of SDR versus MDR phenotypes did not differ among regions ($P = .066$) (Fig 3). Although not significant, the isolates in the south region were characterized by the highest percentage of MDR phenotype (42%, $n = 56/133$), while those from the West region had the lowest percentage (18%, $n = 14/79$) (Fig 3). Differences in MDR phenotype frequencies between tissues were not statistically significant ($P = .664$); however, the highest percentage was among isolates from the skin (44%, $n = 12/27$) and the lowest was among those from the ear (15%, $n = 4/27$) (Fig 4).

The Impact of MDR Phenotypes on the Level of the Resistance

Because MDR was more commonly associated with amoxicillin and enrofloxacin, we compared the level of resistance based on the level of the MICs (ie, low versus

high) for these 2 drugs among the 2 categories of resistance (ie, SDR versus MDR isolates). The MIC geometric mean for amoxicillin was 53 $\mu\text{g}/\text{mL}$ in SDR isolates compared with 381 $\mu\text{g}/\text{mL}$ for MDR isolates ($P < .001$). For enrofloxacin, the MIC geometric mean was 3.6 $\mu\text{g}/\text{mL}$ for SDR compared with 32.2 $\mu\text{g}/\text{mL}$ for MDR. Statistical comparisons were not possible for other drugs because of limited SDR to each drug. The proportion of isolates exhibiting high level resistance to amoxicillin ($\text{MIC} \geq 256 \mu\text{g}/\text{mL}$) was greater (78%; $n = 137/175$) for MDR isolates compared with SDR isolates (5%; $n = 9/175$) (Fig 5A). Similarly, 79% ($n = 58/73$) of MDR isolates expressed high level resistance to enrofloxacin (ie, $\text{MICs} \geq 32 \mu\text{g}/\text{mL}$) compared with only 1% ($n = 1/73$) of SDR isolates (Fig 5B).

PFGE and Clonal Relatedness

The number of isolates subjected to PFGE (ie, phenotypes associated with at least 2 isolates per region) was

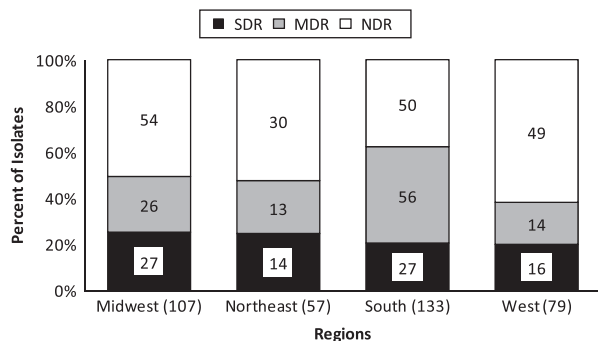


Fig 3. Percentages of SDR, MDR, and NDR phenotypes among *Escherichia coli* clinical isolates by geographical region. For SDR, MDR, and NDR, the percentage was calculated from the total number of isolates in each region. The number of isolates for each phenotype is indicated within each bar, while the numbers in the parentheses after each region represent the total number of isolates in that region. SDR, single drug resistance; MDR, multiple drug resistance; NDR, no drug resistance.

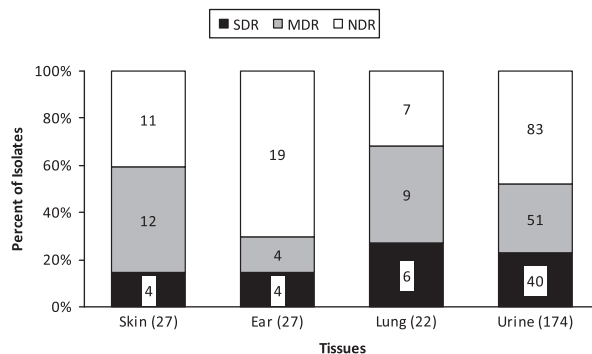


Fig 4. Overall percentage of SDR, MDR, and NDR phenotypes among *Escherichia coli* clinical isolates by tissue site. For SDR, MDR, and NDR, the percentage was calculated from the total number of isolates in that tissue. The number of isolates for each phenotype is indicated within each bar, while numbers in the parentheses after each tissue site represent the total number of isolates in that tissue. Other tissues not represented include abdominal and peritoneal fluid. SDR, single drug resistance; MDR, multiple drug resistance; NDR, no drug resistance.

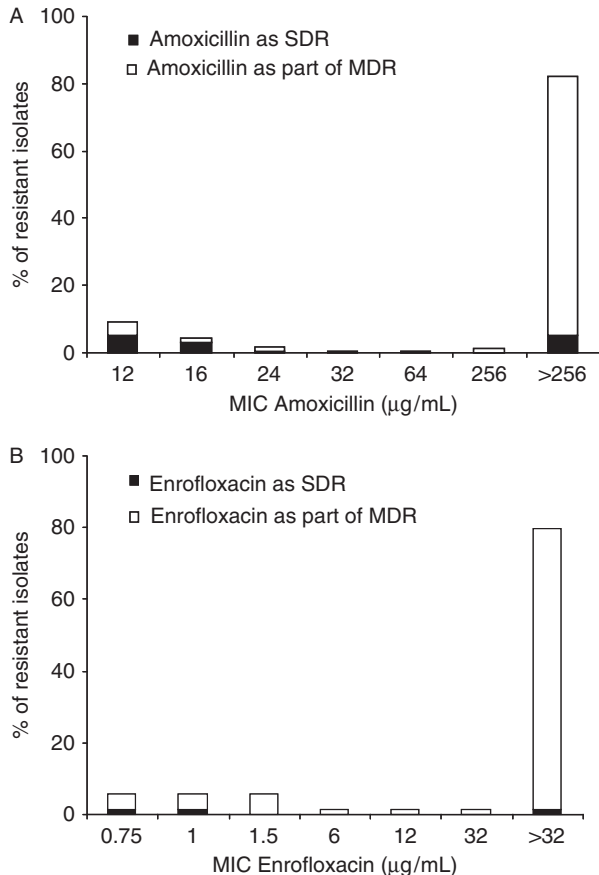


Fig 5. Comparison between SDR and MDR phenotypes based on the MIC for either amoxicillin (A) or enrofloxacin (B) in MDR versus SDR phenotypes. The proportion of MDR compared with SDR isolates with high MIC is greater for both drugs expressing MDR phenotypes. See text for more detail. SDR, single drug resistance; MDR, multiple drug resistance.

South 48, Midwest 27, Northeast 11, and West 5. Of these 91 isolates, 62 different PFGE patterns representing 25 different phenotypes were obtained. Among the isolates from Auburn University ($n = 34$; 11 phenotypes), a total of 18 different PFGE patterns were identified (Fig 6). Antimicrobial resistance phenotypes and genotypes were distinct, thus different PFGE profiles were obtained despite similar antimicrobial resistance profiles among SDR, MDR, and XDR phenotypes. Regarding tissue sites, the largest number of isolates subjected to PFGE were from urine ($n = 46$), with 35 different PFGE patterns representing 15 different phenotypes. Seven different clusters (ie, isolates that were related based on 90% similarity PFGE fingerprinting) were identified. Six clusters from the South and Midwest regions, which comprised 4 clusters with 2 isolates each, and 2 clusters with 4 isolates each, were phenotypically different (Fig 7). Only 1 cluster occurring in the Midwest region, which consisted of 2 isolates, had similar phenotypes.

Discussion

The emergence of antimicrobial resistance among pathogens in human or veterinary medicine increases

the risk of therapeutic failure.¹⁸ In the present study, 57% ($n = 109/193$) of *E. coli* isolates were phenotypically resistant to multiple antimicrobials (Fig 1B). The majority of *E. coli* isolates were genetically distinct from one another based on the analysis of PFGE fingerprint patterns. Our results found no correlation between phenotypes and genotypes within the same region (Fig 6), or within the same sample (ie, urine sample) (Fig 7). This observation is in agreement with studies that demonstrate antibiotic resistance patterns to significantly differ within PFGE types among uropathogenic *E. coli* isolated from dogs.^{19–21} As reported previously, the possibility of horizontal transfer of mobile genetic elements to confer resistance, such as plasmids, integrons, or phage-mediated exchange, is likely to have contributed to different PFGE patterns in our study.^{19–21} The different PFGE profiles might be explained by the large genomic diversity that characterizes *E. coli* isolates. This diversity has also been demonstrated by earlier studies using multilocus enzyme electrophoresis.^{22,23} Identical clones and isolates from similar clonal complexes are frequently recovered from diverse geographical and temporal origins.²⁴ In the case of medically important but numerically small numbers of pathogenic lineages of *E. coli* (ie, enteropathogenic or enterohaemorrhagic strains), recombination (ie, exchange of strands of homologous chromosomal DNA) appears to play a limited role in the genetic diversity. The contribution of recombination to the genetic variability of *E. coli* isolates that produce animal disease has not been fully addressed.²⁴

Many antimicrobials that are used to treat infections in dogs and cats are assumed to increase the selection of resistant strains.¹ The present study revealed a large proportion of resistance to β -lactams, both in SDR and MDR isolates (Fig 1A,B). A similar finding was reported in canine and feline *E. coli* isolates in the United Kingdom during the years 1989–1997, with resistance expressed toward amoxicillin-clavulanic acid, including many MDR isolates.⁵ Similar observations were reported for nonenteric *E. coli* isolates from dogs in the United States between 1990 and 1998, in which isolates exhibited resistance to amoxicillin, carbenicillin, and cephalothin.²⁵ It is tempting to speculate that this magnitude of resistance reflects the popularity of the β -lactams. However, information regarding antimicrobial use among canine and feline practitioners currently is not available.

Our observations indicated that enrofloxacin resistant isolates were more likely to be part of MDR phenotypes compared to SDR phenotypes. This observation is in agreement with previous studies, which also suggest that the selection of fluoroquinolone-resistant mutants of *E. coli* is frequent. Cooke et al²⁰ indicated that enrofloxacin resistant isolates were also MDR to at least 3 other antimicrobials commonly used to treat UTI. Likewise, studies from human medicine have indicated the association of fluoroquinolone use with MDR phenotypes in clinical isolates of uropathogenic *E. coli*.^{26–28} The appearance of MDR phenotypes may be correlated with the use of fluoroquinolones over the recent years in veterinary medicine.²⁸

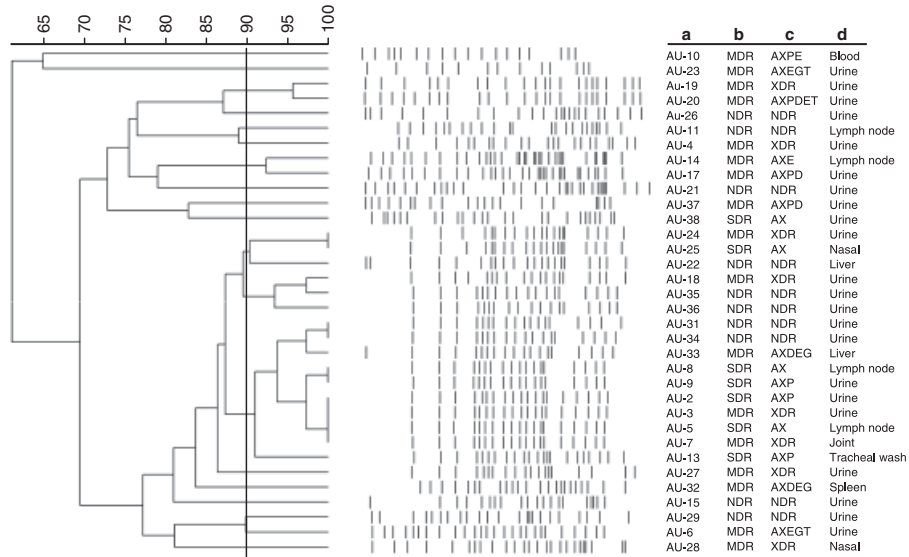


Fig 6. The dendrogram of SDR, MDR, and NDR phenotypes among *Escherichia coli* clinical isolates collected from the same region (South). Isolates from the South were largely submitted from academic institutions. SDR, single drug resistance; MDR, multiple drug resistance; NDR, no drug resistance; XDR, resistance to all 7 antimicrobials; A, amoxicillin; AX, amoxicillin-clavulanic acid; P, cefpodoxime; D, doxycycline; E, enrofloxacin; G, gentamicin; T, trimethoprim-sulfamethoxazole. ^aIsolates' identification. ^bThe SDR and MDR phenotype for each isolate. ^cThe specific antimicrobial resistance phenotype for each isolate. ^dIsolates' tissue site.

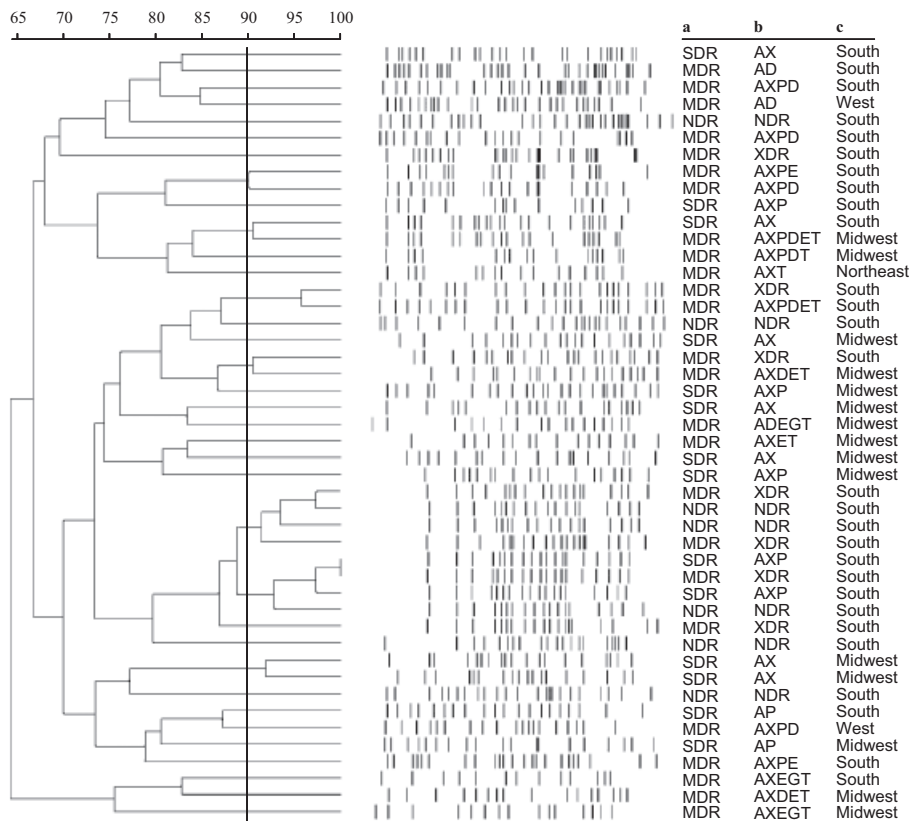


Fig 7. The dendrogram for *Escherichia coli* phenotypes collected from a single tissue (urine). SDR, single drug resistance; MDR, multiple drug resistance; NDR, no drug resistance; XDR, resistance to all 7 antimicrobials; A, amoxicillin; AX, amoxicillin-clavulanic acid; P, cefpodoxime; D, doxycycline; E, enrofloxacin; G, gentamicin; T, trimethoprim-sulfamethoxazole. ^aThe SDR and MDR phenotype for each isolate. ^bThe specific antimicrobial resistance phenotype for each isolate. ^cThe multiple geographical regions of isolates.

We were surprised to find that resistance was greater for isolates collected from the skin compared with other tissues. Increasingly, many homologous resistance genes' sequences have been identified and showed to share high similarities among nonrelated bacteria.^{29,30} The sharing of genes becomes very important in exposed environments, such as the skin, in which the spread of resistance gene can be facilitated among heterogeneous bacterial communities through a variety of mobile elements.³⁰ A higher probability of horizontal gene transfer might explain the proportion and diversity of MDR phenotypes among *E. coli* isolates from skin tissue.³¹

An attribute of our study is the broad distribution of MICs characterizing the study sample population. An advantage of the E test was that both very low and very high MIC (several magnitudes below and above the CLSI susceptible and resistant breakpoints, respectively) could be measured, allowing detection of very susceptible or very resistant isolates. The magnitude of concentrations tested allows us to describe the high level of resistance that is associated with MDR isolates compared with SDR isolates. It may be important to understand that MDR isolates are not only resistant to many drugs, but the level of resistance to those drugs is very high and thus might not likely to be overcome by increasing doses, nor combination antimicrobial therapy.

Our study was confined to a restricted time frame (summer 2005), a limited sample size, and a limited number of antimicrobial drugs to which the isolates were tested. However, the chosen drugs represented all drug classes commonly used by veterinarians. Further, geographical regions of collection were broad and the isolates were collected from multiple tissue sites. As such, isolates tested should represent a broad diversity of those causing infection in dogs and cats. However, among the limitations in this diversity are the confounding factors of region and laboratory type. In our study, the magnitude of resistance was greater in isolates submitted from the South and less in isolates submitted from the West compared with the Midwest and Northeast. However, submitting laboratories from the South were largely academic in location while those from the West were commercial. It is likely, but can not be proven, that more isolates submitted from academic laboratories were sampled from animals in a secondary or tertiary care setting compared with those submitted from commercial laboratories. Isolates from the former may be more likely to be associated with previous antimicrobial use. We could not separate out the confounding factors of region or laboratory type. More importantly, we had no information on the patients from which our isolates were collected regarding previous antimicrobial use, including the appropriateness of dosing regimens; both factors might contribute to antimicrobial resistance.³² Further studies (eg, surveillance) are indicated to identify those risk factors associated with resistance.

In conclusion, SDR and MDR phenotypes identified in this study were not associated with any particular PFGE pattern within the same sample or among the same geographical location collected over the same period. Further, MDR phenotypes were more commonly

isolated in the South regions, although this may reflect the academic laboratory base of this region in our study. Enrofloxacin resistance was rarely associated with SDR, but frequently associated with MDR phenotypes. Most importantly, MDR phenotypes were associated with high level of resistance to each of the drugs in the isolate resistant phenotype. This study underscores the need for a comprehensive assessment of antimicrobial resistance in dogs and cats, including factors that increase the risk of emergent resistance.

Footnotes

^a Shaheen BW, Boothe DM, Oyarzabal OA, et al. Characterization of clinical *Escherichia coli* isolates expressing multidrug resistance recovered from canine and feline with spontaneous disease. Am Coll Vet Intern Med Ann Forum, San Antonio, TX, 2008 (abstract)

^b Remel/ Thermo Fisher Scientific; Lenexa, KS

^c Epsilon; AB Biodisk, Solna, Sweden

^d American Tissue Cell Culture, Manassas, VA

^e Minitab 15, statistical software, State College, PA

^f Polypropylene tubes; 30 by 115 mm; Becton Dickinson, Franklin Lakes, NJ

^g *Xba*I, restriction enzyme; Roche Applied Science, Indianapolis, IN

^h CHEF Mapper XA; Bio-Rad Laboratories, Hercules, CA

ⁱ Gel Doc imaging system; Syngene, Frederick, MA

^j GeneSnap; Syngene

^k BioNumerics software; Applied Maths, Sint-Martens-Latem, Belgium

Acknowledgments

We thank Robert S. Miller, Department of Poultry Science, Auburn University, for his technical assistance. This study was supported, in part, by a grant-in-aid from the Waltham Foundation.

References

1. von Baum H, Marre R. Antimicrobial resistance of *Escherichia coli* and therapeutic implications. Int J Med Microbiol 1995;295:503–514.
2. Normand EH, Gibson RN, Taylor DJ, et al. Trends of antimicrobial resistance in bacterial isolates from a small animal referral hospital. Vet. Rec 2000;146:151–155.
3. Guardabassi L, Schewarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 2004;54:321–332.
4. Cohn LA, Gary AT, Fales WH, et al. Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. J Vet Diagn Invest 2003;15:338–343.
5. Normand EH, Gibson NR, Reid SW, et al. Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. Prev Vet Med 2000;46:267–278.
6. Sanchez S, McCrackin Stevenson MA, Hudson CR, et al. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. J Clin Microbiol 2002;40:3586–3595.
7. Beutin L. *Escherichia coli* as a pathogen in dogs and cats. Vet Res 1999;30:285–298.

8. Johnson JR, Delavari P, Stell AL, et al. Molecular comparison of extraintestinal *Escherichia coli* isolates of the same electrophoretic lineages from humans and domestic animals. *J Infect Dis* 2001;183:154–159.
9. Johnson JR, Clabots C, Kuskowski MA. Multiple-host sharing, long-term persistence, and virulence of *Escherichia coli* clones from human and animal household members. *J Clin Microbiol* 2008;46:4078–4082.
10. Johnson JR, Stell AL, Delavari P, et al. Phylogenetic and pathotypic similarities between *Escherichia coli* isolates from urinary tract infections in dogs and extraintestinal infections in humans. *J Infect Dis* 2001;183:897–906.
11. Starcic M, Johnson JR, Stell AL, et al. Haemolytic *Escherichia coli* isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxicogenic strains. *Vet Microbiol* 2002;85:361–377.
12. Johnson JR, Stell AL, Delavari P. Canine feces as a reservoir of extraintestinal pathogenic *Escherichia coli*. *Infect Immun* 2001;69:1306–1314.
13. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard, CLSI document M31-A2, 2nd ed. Wayne, PA: National Committee for Clinical Laboratory Standards; 2002:22–57.
14. Livermore DM, James D, Reacher M, et al. Trends in fluoroquinolone (ciprofloxacin) resistance in Enterobacteriaceae from bacteremias, England and Wales, 1990–1999. *Emerg Infect Dis* 2002;8:473–478.
15. Kelesidis T, Karageorgopoulos DE, Kelesidis I, et al. Tigecycline for the treatment of multidrug-resistant Enterobacteriaceae: A systematic review of the evidence from microbiological and clinical studies. *J Antimicrob Chemother* 2008;62:895–904.
16. Barrett TJ, Lior H, Green JH, et al. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157: H7 by using pulsed-field gel electrophoresis and phage typing. *J Clin Microbiol* 1994;32:3013–3017.
17. Hunter SB, Vauterin P, Lambert-Fair MA, et al. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: Converting the national databases to the new size standard. *J Clin Microbiol* 2005;43:1045–1050.
18. Costa D, Poeta P, Sáenz Y, et al. Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. *Vet Microbiol* 2007;127:97–105.
19. Drazenovitch N, Ling GV, Foley J. Molecular investigation of *Escherichia coli* strains associated with apparently persistent urinary tract infection in dogs. *J Vet Intern Med* 2004;18:301–306.
20. Cooke CL, Singer RS, Jang SS, et al. Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. *J Am Vet Med Assoc* 2002;220:190–192.
21. Mentula S, Virtanen T, Kanervo-Nordström A, et al. Relatedness of *Escherichia coli* strains with different susceptibility patterns isolated from beagle dogs during ampicillin treatment. *Int J Antimicrob Agents* 2006;27:46–50.
22. Milkman R. Electrophoretic variation in *Escherichia coli* from natural sources. *Science* 1973;182:1024–1026.
23. Milkman R. Recombination and population structure in *Escherichia coli*. *Genetics* 1997;146:745–750.
24. Feil EJ, Spratt BG. Recombination and the population structures of bacterial pathogens. *Annu Rev Microbiol* 2001;55:561–590.
25. Oluoch AO, Kim CH, Weisiger RM, et al. Nonenteric *Escherichia coli* isolates from dogs: 674 cases (1990–1998). *J Am Vet Med Assoc* 2001;218:381–384.
26. Karlowsky LJ, Kelly C, Thornsberry ME, et al. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Antimicrob Agents Chemother* 2002;46:2540–2545.
27. Karlowsky JA, Thornsberry C, Jones ME, et al. Susceptibility of antimicrobial-resistant urinary *Escherichia coli* isolates to fluoroquinolones and nitrofurantoin. *Clin Infect Dis* 2003;36:183–187.
28. Cooke CL, Singer RS, Jang SS, et al. Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. *J Am Vet Med Assoc* 2002;15:190–192.
29. Aarestrup FM. The origin, evolution, and local and global dissemination of antimicrobial resistance. In: Aarestrup FM, ed. *Antimicrobial Resistance in Bacteria of Animal Origin*. Washington, DC: ASM Press; 2006:339–360.
30. Dionisio F, Matic I, Radman M, et al. Plasmids spread very fast in heterogeneous bacterial communities. *Genetics* 2002;162:1525–1532.
31. Osborn AM, Böltner D. When phage, plasmids, and transposons collide: Genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. *Plasmid* 2002;48:202–212.
32. Boothe DM, Boeckh A, Simpson RB, et al. Comparison of pharmacodynamic and pharmacokinetic indices of efficacy for 5 fluoroquinolones toward pathogens of dogs and cats. *J Vet Intern Med* 2006;20:1297–1306.