Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections

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Objective—To assess the strain heterogeneity of enrofloxacin-resistant *Escherichia coli* associated with urinary tract infections in dogs at a veterinary medical teaching hospital (VMTH). In addition, strains from other veterinary hospitals in California were compared with the VMTH strains to assess the geographic distribution of specific enrofloxacin-resistant *E coli* isolates.

Design—Bacteriologic study.

Sample Population—56 isolates of *E coli* from urine samples (43 isolates from dogs at the VMTH, 13 isolates from dogs from other veterinary clinics in California).

Procedures—Pulsed field gel electrophoresis was performed on 56 isolates of *E coli* from urine samples from 56 dogs. All 56 isolates were tested for susceptibility to amoxicillin, chloramphenicol, enrofloxacin, tetracycline, trimethoprim-sulphamethoxazole, cephalexin, and ampicillin. Enrofloxacin usage data from 1994 to 1998 were obtained from the VMTH pharmacy.

Results—Several strains of enrofloxacin-resistant *E coli* were collected from urine samples from the VMTH, and strains identical to those from the VMTH were collected from other veterinary clinics in California. For the isolates that did share similar DNA banding patterns, variable antibiotic resistance profiles were observed.

Conclusions and Clinical Relevance—The increased occurrence of enrofloxacin-resistant *E coli* from urine samples from dogs at the VMTH was not likely attributable to a single enrofloxacin-resistant clone but may be attributed to a collective increase in enrofloxacin resistance among uropathogenic *E coli* in dogs in general. (*J Am Vet Med Assoc* 2002;220:190–192)

Enrofloxacin, a fluoroquinolone antibiotic that was approved by the Food and Drug Administration in 1989 for veterinary use in dogs, has a broad spectrum of activity against many gram-negative and gram-positive bacteria that are associated with **urinary tract infections (UTI)**.[1] Fluoroquinolone antibiotics block bacterial DNA replication by inhibiting the prokaryotic DNA supercoiling enzyme DNA gyrase.

The primary mechanism of bacterial resistance to fluoroquinolone antibiotics has been described in *Escherichia coli* as a chromosomal mutation in *gyr*A, the gene encoding the A subunit of the enzyme DNA gyrase.[2-5] Specific mutations have been identified in the quinolone resistance determining region of this gene that alter the enzyme such that it does not bind as well to the quinolone, and thus the antibiotic is unable to inhibit the enzyme effectively.[2] Topoisomerase IV (encoded by the *par*C and *par*E genes) is a secondary target of the fluoroquinolone antibiotics, and mutations in *par*C and *parE* have also been shown to contribute to quinolone resistance.[2,5,6] Decreased permeability to the quinolones as well as decreased drug accumulation have also been reported to contribute to quinolone resistance.[2,4,7,8]

Beginning in late 1996, there was an increase in the number of cases in which enrofloxacin-resistant *E coli* were isolated from the urine of dogs with UTI at a **veterinary medical teaching hospital (VMTH)**. It was not known whether these *E coli* represented an enrofloxacin-resistant strain occurring within the VMTH or if they reflected a collective increase in enrofloxacin resistance among uropathogenic *E coli* in dogs in general.

We hypothesized that the increased prevalence of enrofloxacin-resistant *E coli* associated with UTI in dogs at the VMTH was attributable to a collective increase in enrofloxacin resistance among uropathogenic *E coli* in dogs. Therefore, the purpose of the study reported here was to use **pulsed-field gel electrophoresis (PFGE)** to assess the strain heterogeneity of enrofloxacin-resistant *E coli* associated with UTI in dogs at the VMTH and to compare these isolates with strains from other veterinary hospitals in California.

Materials and Methods

Collection of bacterial isolates—Forty-three isolates of *E coli* were obtained from the urine of dogs that were evaluated between September 1996 and September 1998 at the VMTH; the dogs had UTI and, in several instances, various other maladies. Of these 43 *E coli* isolates, 23 were resistant to enrofloxacin, and 20 were susceptible. In addition, 13 enrofloxacin-resistant *E coli* isolates that were obtained from the urine of dogs at other veterinary hospitals in California were included for comparison. It is unknown whether the dogs from which these isolates originated had any contact with the VMTH.

Antibiotic susceptibility testing—All isolates obtained from the VMTH were tested for susceptibilities to antimicrobial agents by the microbroth dilution method.[a] The assay was performed as described by the manufacturer, and appropriate quality control organisms were included. The specific breakpoints (as set by the **US National Committee for Clinical Laboratory Standards [NCCLS]**[9]) used in this study to determine susceptibility were as follows: ampicillin, ≥ 128 µg/ml; amoxicillin-clavulanic acid, ≥ 128 µg/ml; chloramphenicol, ≥ 32 µg/ml; cephalexin, ≥ 64 µg/ml; tetracy-

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cline, ≥ 128 µg/ml; and trimethoprim-sulphamethoxazole, ≥ 32 µg/ml. Isolates obtained from sources outside of the VMTH were tested by the disk diffusion method.[10] Isolates were categorized as susceptible, intermediate, or resistant based on the NCCLS guidelines.[9]

Pulsed field gel electrophoresis—Pulsed field gel electrophoresis was performed according to manufacturer's recommendations.[b] Briefly, whole cell DNA was isolated in agarose blocks and digested with the restriction endonuclease *Xba*I.[c] The resulting DNA fragments were separated in a 1.2% agarose gel by use of a contour-clamped homogeneous electric field PFGE apparatus.[b] Gels were run for 22 hours at 180 V and an electrical field angle of 120° at 14 C, with pulse times linearly increasing from 1 to 40 seconds. A mixture of concatemers of λcI857Sam7[b] was run on each gel as a molecular weight standard. After electrophoresis, the electrophoretograms were stained with ethidium bromide, digitized into a computer,[b] and analyzed.[b] Genetic similarity between each pair of isolates was assessed by use of the Dice coefficient of similarity.[11] A dendrogram was created with an unweighted pair group method, using average linkages clustering.

Relationship between enrofloxacin usage and enrofloxacin resistance—Data regarding the number of grams of enrofloxacin purchased by the VMTH between 1994 and 1998 were obtained from the VMTH pharmacy. The proportion of *E coli* isolates collected from urine samples in dogs between 1994 and 1998 that were resistant to enrofloxacin was retrieved from records at the VMTH. Care was taken to count only 1 isolate of *E coli* from each dog. The annual change in the proportion of *E coli* isolates collected from urine samples in dogs at the VMTH that were resistant to enrofloxacin was assessed by use of a χ^2 test.[d] Values of $P < 0.05$ were considered significant.

Results

Pulsed field gel electrophoresis—Analysis of electrophoretograms revealed that DNA banding patterns from 16 of the 36 resistant isolates were similar (> 90% similarity). However, 4 of these 16 isolates were collected from outside the VMTH. The 20 remaining resistant isolates were somewhat heterogeneous, except in a few instances in which 2, 3, or 4 isolates shared the same DNA banding patterns. All of the enrofloxacin-susceptible isolates had unique DNA banding patterns.

Susceptibility testing—All 56 isolates were tested for susceptibility to amoxicillin, chloramphenicol, enrofloxacin, tetracycline, trimethoprim-sulphamethoxazole, cephalexin, and ampicillin (**Table 1**).

Table 1—Resistance profiles of enrofloxacin-resistant *Escherichia coli* isolated from urine samples from dogs with urinary tract infections evaluated at a veterinary medical teaching hospital

	Resistance pattern*					
No. of isolates	A	A/C	C	Cp	T	T/S
15	X		X	X	X	X
6	X	X	X	X	X	X
6	X		X		X	X
3	X		X	X		X
4	X		X	X	X	
1	X		X	X		
1	X			X		X

*X = Resistant. A = Ampicillin. A/C = Amoxicillin-clavulanic acid. C = Chloramphenicol. Cp = Cephalexin. T = Tetracycline. T/S = Trimethoprim-sulphamethoxazole.

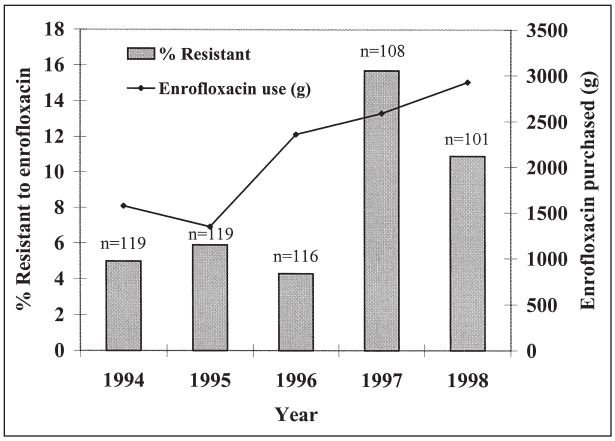

Figure 1—Percentage of *Escherichia coli* isolated from urine samples from dogs and resistant to enrofloxacin versus grams of enrofloxacin purchased per year by the veterinary medical teaching hospital pharmacy from 1994 through 1998.

Many of these isolates were resistant to more than 1 antibiotic. More than 1 antibiotic resistance profile was present within each cluster of genotypically similar isolates (isolates with > 90% similarity).

Relationship between enrofloxacin usage and enrofloxacin resistance—The change in the proportion of *E coli* isolates collected from urine in dogs at the VMTH that were resistant to enrofloxacin between 1994 and 1996 was not significant ($P > 0.60$). However, between 1996 and 1997, there was a significant increase ($P < 0.005$) in the proportion of enrofloxacin-resistant *E coli* isolated from urine samples from dogs with UTI. The decrease in the proportion of enrofloxacin-resistant *E coli* isolated from urine samples between 1997 and 1998 was not significant ($P > 0.30$). The increase in enrofloxacin resistance in 1997 followed an increase in enrofloxacin usage at the VMTH from 1,334.4 g in 1995 to 2,358.3 g in 1996 (**Fig 1**).

Discussion

We have not ruled out the possibility that the VMTH was a point of dissemination of an enrofloxacin-resistant strain of *E coli*. However, many different strains of enrofloxacin-resistant *E coli* were collected from the VMTH, and within the clusters of similar resistant isolates, more than 1 antibiotic resistance profile was evident. Therefore, the increased prevalence of enrofloxacin-resistant *E coli* in urine from dogs with UTI at the VMTH was not likely attributable to a single enrofloxacin-resistant clone.

Several investigators have associated increased antibiotic usage with increased antibiotic resistance.[3,12,13] The total amount of enrofloxacin purchased by the VMTH pharmacy almost doubled between 1994 and 1998, with the largest increase occurring between 1995 and 1996 (Fig 1). In the data collected from 1997, we observed a significant increase in the number of enrofloxacin-resistant *E coli* isolated in urine from dogs with UTI at the VMTH. If enrofloxacin usage at other veterinary clinics increased as it had at the VMTH, this increased usage might select for enrofloxacin-resistant bacteria.

Another concern is the multiple antibiotic resistance accompanying enrofloxacin resistance in the isolates in this study. Each enrofloxacin-resistant isolate in this study was also resistant to at least 3 other antibiotics that are commonly used to treat UTI. Further characterization of the mechanisms of resistance to enrofloxacin as well as to the other antibiotics assessed in this study is needed.

Monitoring antibiotic usage and resistance patterns in a VMTH may serve as an early indicator of changes in antibiotic susceptibility of clinical isolates. Only through the molecular characterization of the *E coli* isolated in this study were we able to detect that the increase in enrofloxacin-resistant *E coli* at the VMTH was not likely to have been attributable to the dissemination of a single resistant clone. A system within the VMTH whereby resistance trends are documented and specific isolates are genotyped would enhance the monitoring potential of the VMTH.

[a]Trek Diagnostic Systems Inc, Westlake, Ohio.
[b]BioRad, Hercules, Calif.
[c]New England Bio Labs, Beverly, Mass.
[d]EpiInfo, Centers for Disease Control, Atlanta, Ga.

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