

Antimicrobial resistance of *Escherichia coli* isolates from canine urinary tract infections

Shao-Kuang CHANG¹⁾, Dan-Yuan LO²⁾, Hen-Wei WEI³⁾ and Hung-Chih KUO^{2)*}

¹⁾Graduate Institute of Veterinary Medicine, National Taiwan University, Taipei 106, Taiwan, ROC

²⁾Department of Veterinary Medicine, National Chiayi University, Chiayi, 600, Taiwan, ROC

³⁾Department of Animal Science and Technology, National Taiwan University, Taipei 106, Taiwan, ROC

(Received 1 June 2013/Accepted 17 September 2014/Published online in J-STAGE 28 October 2014)

ABSTRACT. This study determined the antimicrobial resistance profiles of *Escherichia coli* isolates from dogs with a presumptive diagnosis of urinary tract infection (UTI). Urine samples from 201 dogs with UTI diagnosed through clinical examination and urinalysis were processed for isolation of *Escherichia coli*. Colonies from pure cultures were identified by biochemical reactions ($n=114$) and were tested for susceptibility to 18 antimicrobials. The two most frequent antimicrobials showing resistance in Urinary *E. coli* isolates were oxytetracycline and ampicillin. Among the resistant isolates, 17 resistance patterns were observed, with 12 patterns involving multidrug resistance (MDR). Of the 69 tetracycline-resistant *E. coli* isolates, *tet(B)* was the predominant resistance determinant and was detected in 50.9% of the isolates, whereas the remaining 25.5% isolates carried the *tet(A)* determinant. Most ampicillin and/or amoxicillin-resistant *E. coli* isolates carried *bla*_{TEM-1} genes. Class 1 integrons were prevalent (28.9%) and contained previously described gene cassettes that are implicated primarily in resistance to aminoglycosides and trimethoprim (*dfpA1*, *dfpA17-aadA5*). Of the 44 quinolone-resistant *E. coli* isolates, 38 were resistant to nalidixic acid, and 6 were resistant to nalidixic acid, ciprofloxacin and enrofloxacin. Chromosomal point mutations were found in the GyrA (Ser83Leu) and ParC (Ser80Ile) genes. Furthermore, the aminoglycoside resistance gene *aacC2*, the chloramphenicol resistant gene *cmlA* and the florfenicol resistant gene *floR* were also identified. This study revealed an alarming rate of antimicrobial resistance among *E. coli* isolates from dogs with UTIs.

KEY WORDS: antimicrobial resistance, class 1 integrons, urinary *Escherichia coli*

doi: 10.1292/jvms.13-0281; *J. Vet. Med. Sci.* 77(1): 59–65, 2015

Escherichia coli (*E. coli*) is the most important causative bacterium of urinary tract infections (UTIs) in both humans and dogs, and strains of these species are often abundant in the gastrointestinal tract at the time of infection [7, 24, 26, 29]. In past studies of canine UTIs, the majority of bacterial isolates were *E. coli* (33–56%) [2, 12, 20, 29, 34, 39]. Historically, a range of antimicrobial agents has been used to treat UTIs in veterinary medicine, including penicillins, cephalosporins, tetracyclines, chloramphenicols, aminoglycosides, fluoroquinolones and potentiated sulfonamides. The use of antimicrobial drugs has been associated with an increasing trend of antimicrobial resistance among canine *E. coli* isolates over the last decade [33]. Thus, the management of UTIs in dogs has become more complicated, as the prevalence of antibiotic-resistant strains of *E. coli* has increased. In addition, the frequent close physical contact between dogs and humans increases the potential for transmission of resistant bacteria between companion animals and humans, as well as the potential for exchange or transfer of antimicrobial-resistant genes to human pathogens [22]. The objectives of this study were to determine (i) the frequency of canine uro-

pathogenic *E. coli* at the National Taiwan University Veterinary Hospital (NTUVH) and the National Chiayi University Veterinary Teaching Hospital (NCYUVTH), (ii) the percentage of antimicrobial resistance and (iii) the distribution and mechanisms of bacterial resistance to β -lactams, chloramphenicols, tetracyclines, quinolones, aminoglycosides and sulfamethoxazole-trimethoprim. The results should provide useful information for veterinarians in the management of persistent or recurrent UTI in dogs.

MATERIALS AND METHODS

Sampling methods: Urine samples were obtained from 201 non-medicated adult dogs (aged >1 y) of both sexes with a presumptive diagnosis of UTI from July 2010 to June 2011. The inclusion criteria included clinical signs of UTI, such as hematuria and dysuria, urinalysis results that included red blood cell counts >5 under a high-power field (HPF) and proteinuria, and >10³ colony-forming units (CFU) of bacteria per milliliter of urine at the first plating. Urine samples were obtained by catheterization after thorough cleansing of the genital area.

Bacterial isolation, culture conditions and identification: The fresh urine samples were refrigerated temporarily and were cultured using a calibrated pipette to deliver 10 μ l and 100 μ l of samples onto Columbia agar supplemented with 5% sheep blood and onto MacConkey agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, U.S.A.) upon receipt. The blood agar plates were incubated with

*CORRESPONDENCE TO: KUO, H. J., Department of Veterinary Medicine, National Chiayi University, No. 580, Xinmin Rd., Chiayi City 60054, Taiwan. e-mail: hjkuo@mail.ncyu.edu.tw

©2015 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

5% CO₂, and the MacConkey agar plates were incubated aerobically. All samples were incubated at 37°C for 18 to 24 hr until adequate growth was present. Primary plates were carefully inspected for colonies of *E. coli*, which were plated onto sheep blood agar plates; these plates were incubated at 37°C for 24 hr. Suspected colonies were identified as *E. coli* using standard techniques, including indole, Methyl red–Voges-Proskauer (MR–VP) and citrate biochemical testing and analysis with an API-20E system (bioMérieux, Marcy l’Etoile, France) [17].

Antimicrobial susceptibility testing: Susceptibility was tested quantitatively by broth microdilution with cation-adjusted Mueller–Hinton broth, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Eight drug classes were included in this study. These classes represented the most frequently used antibiotics for cases of UTI in our community. Tetracyclines were represented by oxytetracycline and doxycycline. Aminoglycosides were represented by gentamicin and amikacin. Quinolones were represented by nalidixic acid, enrofloxacin and ciprofloxacin. Penicillins were represented by the amoxicillin-clavulanic acid combination, amoxicillin and ampicillin. Lastly, cephalosporins were represented by cefazolin, ceftiofur, ceftazidime and cefotaxime. Sulfamethoxazole-trimethoprim, colistin, florfenicol and chloramphenicol were also tested. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

Bacterial DNA preparation, PCR assays and DNA sequencing: PCR analyses for preselected resistant genes were performed for all of the isolates. The total DNA of *E. coli* isolates was extracted by using an InstaGene DNA Purification Matrix kit (Bio-Rad, Hercules, CA, U.S.A.) according to the manufacturer’s instructions. PCR primers specific to these resistant genes were designed based on the gene sequence information from previously published studies [5, 28, 32, 42]. PCR was performed in a 20 µl mixture containing 1 µl of template DNA, 0.5 µl of each primer (10 nmol l⁻¹), 10 µl of 2× PCR Mix (Fermentas, MBI) and 8 µl of ddH₂O. PCR was performed using 30 cycles of denaturation at 94°C and amplification at 72°C for 10 min. Amplicons were visualized by electrophoresis at 100 V in a 2% agarose gel. Amplified PCR products were purified with a QIAquick PCR purification Kit (Qiagen, Valencia, CA, U.S.A.) and sequenced by ABI 3730 × 1 capillary sequencers (Applied Biosystems, Foster City, CA, U.S.A.). The DNA sequences were compared using the BLAST online search engine from GenBank at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/blast>) [1].

The *gyrA*, *gyrB*, *parC* and *parE* genes were amplified by PCR using the primers and PCR conditions described previously [16]. Both strands of the purified PCR products were sequenced, and the results were compared with the sequences of wild-type *E. coli gyrA* (NCBI X06373), *gyrB* (P06982), *parC* (P20082) and *parE* (P20083). Comparisons were performed using NCBI BLAST, the ClustalW Multiple Sequence Alignment program and the Lasergene sequence analysis software package (version 4.0, DNASTAR, Madison, WI, U.S.A.) [44].

Statistical analysis: The statistical tests used were the chi-square test and Fisher’s exact test, which were performed using the Mixed Procedure in SAS (version 8.2; SAS Institute, Inc., Cary, NC, U.S.A.). *P* < 0.05 was considered significant.

RESULTS

Bacterial isolates and antimicrobial susceptibility testing: A total of 114 *E. coli* strains were isolated from 201 urine samples from dogs with suspected urinary tract infections. Among them, 69 isolates were collected at the NTUVH, and 45 isolates collected at the NCYUVTH. The minimum inhibitory concentrations at which 50% and 90% of the isolates were inhibited (MIC₅₀ and MIC₉₀) and the antimicrobial susceptibility profiles for the different antimicrobial agents are summarized in Table 1. The percentages of *E. coli* strains resistant to oxytetracycline, ampicillin, amoxicillin, nalidixic acid, sulfamethoxazole-trimethoprim, chloramphenicol, florfenicol and doxycycline were 60.5% (69/114), 50% (57/114), 44.7% (51/114), 38.6% (44/114), 34.2% (39/114), 31.6% (36/114), 31.6% (36/114) and 28.9% (33/114), respectively. The lowest levels of resistance were found for gentamicin (10.5%, 12/114), enrofloxacin (5.3%, 6/114), ciprofloxacin (5.3%, 6/114) and amoxicillin-clavulanic acid (2.6%, 3/114). Resistance to cephalosporins (cefazolin, ceftazidime, cefotaxime and ceftiofur), amikacin or colistin was not detected. Resistance to ampicillin, amoxicillin, chloramphenicol, oxytetracycline and sulfamethoxazole-trimethoprim was significantly higher in the isolates from NTUVH (43.5–73.9%) than in those from NCYUVTH (13.3–40.0%) (*P* < 0.05). Isolates from NCYUVTH were significantly more resistant to ciprofloxacin and enrofloxacin (13.3%) than those from NTUVH (0%) (*P* < 0.01).

Resistance to three or more antimicrobials (multiresistance) was observed in 52.6% (60/114) of the isolates from the urine samples (Table 2). None of the isolates were resistant to all of the antimicrobials tested. Resistance to oxytetracycline, amoxicillin and ampicillin was the most common combination in the urine samples and was observed in 42 (36.8%) of the 114 isolates.

Detections of antibiotic resistance genes: A total of 69 tetracycline-resistant isolates were screened by PCR and sequencing for the 5 tetracycline determinants, *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)* and *tet(E)*. These results are shown in Table 3. Of the tetracycline-resistant isolates, 79.7% (55/69) were positive by PCR for *tet* genes, with *tet(B)* being the most prevalent gene (50.9%, 28/55), followed by *tet(A)* (25.5%, 14/55), *tet(A+B)* (14.5%, 8/55), *tet(B+C)* (5.5%, 3/55) and *tet(A+B+C)* (3.6%, 2/55); *tet(D)* and *tet(E)* were not detected in any isolates.

The presence of the *cmlA* and *floR* genes was investigated in the isolates resistant to chloramphenicol and/or florfenicol; of 36 chloramphenicol- and florfenicol-resistant isolates, all were found to harbor *cmlA*, and 18 had *floR* genes. The frequency of detection of the *cmlA* gene was greater than that of the *floR* gene. Our results also showed that three *E. coli* isolates (8.3%, 3/36) were carrying both the *floR* and *cmlA* genes.

Table 1. Antimicrobial susceptibility profiles of uropathogenic *Escherichia coli*

Class	Antimicrobial agents (mg/l)	NTUVH (n=69)			NCYUVTH (n=45)			Overall <i>E. coli</i> (n=114)		
		MIC ₅₀	MIC ₉₀	R% ^{a,b}	MIC ₅₀	MIC ₉₀	R% ^{a,b}	MIC ₅₀	MIC ₉₀	R% ^{a,b}
Aminoglycosides	Amikacin	2	8	0	4	8	0	2	8	0
	Gentamicin	1	2	8.7	1	64	13.3	1	64	10.5
Cephalosporins	Cefazolin	2	16	0	2	8	0	2	8	0
	Cefotaxime	1	1	0	1	2	0	1	2	0
	Ceftazidime	2	2	0	1	2	0	2	2	0
	Ceftiofur	1	1	0	0.5	1	0	0.5	1	0
Penicillins	Ampicillin	512	>1024	60.9	16	1024	33.3	16	>1024	50
	Amoxicillin	>1024	>1024	56.5	8	>1024	26.7	16	>1024	44.7
	Amoxicillin-clavulanic acid	8/4	16/8	0	8/4	16/8	6.7	8/4	16/8	2.6
Phenicol	Chloramphenicol	16	512	43.5	8	32	13.3	8	512	31.6
	Florfenicol ^{c)}	4	8	34.8	4	16	26.7	4	16	31.6
Polymyxins	Colistin	1	1	0	1	2	0	1	2	0
Quinolones	Ciprofloxacin	<0.125	0.25	0	<0.125	32	13.3	0.125	0.25	5.3
	Enrofloxacin ^{c)}	0.25	0.5	0	<0.125	32	13.3	0.125	1	5.3
	Nalidixic acid	8	512	43.5	8	>1024	33.3	8	1024	38.6
Sulfonamides	Sulfamethoxazole-trimethoprim	4/76	>1024	43.5	1/19	>1024	20	2/38	>1024	34.2
Tetracyclines	Doxycycline	8	128	39.1	2	32	26.7	4	128	28.9
	Oxytetracycline	256	1024	73.9	8	1024	40	8	1024	60.5

a) MIC breakpoints according to CLSI guidelines. b) Percentage of resistant isolates. c) Because the MIC breakpoints for UTIs were not provided in the 2007 CLSI guidelines, the values for bovine respiratory pathogens were used [10].

Table 2. Distribution of antimicrobial drug resistance patterns among the 114 uropathogenic *E. coli* strains

Resistance pattern	No. of isolates
AMP	3
AUG	3
FLO	9
NA	5
CHL, FLO	6
AMP, NA, SUL	3
CHL, NA, OTC	3
AMO, AMP, CHL, OTC	9
AMO, AMP, GEN, SUL	6
DOX, GEN, NA, OTC	3
AMO, AMP, CHL, FLO, OTC	3
AMO, AMP, CIP, EN, NA	3
AMO, AMP, DOX, NA, OTC, SUL	9
AMO, AMP, CHL, DOX, GEN, OTC, SUL	3
AMO, AMP, DOX, FLO, NA, OTC, SUL	6
AMO, AMP, CHL, DOX, FLO, NA, OTC, SUL	9
AMO, AMP, CHL, CIP, DOX, EN, FLO, NA, OTC, SUL	3

AMO, amoxicillin; AMP, ampicillin; AUG, amoxicillin-clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; DOX, doxycycline; EN, enrofloxacin; FLO, florfenicol; GEN, gentamicin; NA, nalidixic acid; OTC, oxytetracycline; SUL, sulfamethoxazole-trimethoprim.

The fluoroquinolone-resistant isolates (n=6) had 2 point mutations, one in GyrA (Ser-83 to Leu) and one in ParC (Ser-80 to Ile) (Table 3). No mutations were detected in the known resistance regions of GyrB or ParE. Nalidixic acid-resistant isolates (n=44) with a low level of MICs for ciprofloxacin (<0.125–0.25 mg/l) and enrofloxacin (<0.125–1 mg/l) harbored a single mutation in GyrA (Ser-83

to Leu). In this study, the aminoglycoside acetyltransferase variant gene (*aac* (6')-Ib-cr) and active drug efflux pump genes (*qnrA*, *qnrB*, *qnrS* and *qepA*) were not detected in any isolate.

PCRs with primers specific for the *bla*_{TEM}, *bla*_{SHV} and *bla*_{AmpC} genes were performed for the 57 ampicillin- and/or amoxicillin-resistant isolates in this study, and the results are

Table 3. Prevalence of antimicrobial resistance genes among 114 canine *E. coli* isolates from UTIs

Antimicrobial agents	Resistance gene (s)	No. of positive isolates
Aminoglycosides	<i>aadA5</i>	18
	<i>aadB</i>	0
	<i>aacC2</i>	10
Amoxicillin/ampicillin	<i>bla</i> _{TEM-1b}	40
	<i>bla</i> _{SHV-1}	14
	<i>bla</i> _{AmpC}	0
Chloramphenicols	Only <i>cmlA</i>	33
	Only <i>floR</i>	15
	<i>cmlA</i> + <i>floR</i>	3
Ciprofloxacin/enrofloxacin	<i>gyrA</i> 83 (Ser-Leu)	6
	<i>parC</i> 80 (Ser-Ile)	6
	<i>aac</i> (6')-Ib-cr	0
	<i>qnrA</i> , <i>qnrB</i> and <i>qnrS</i>	0
	<i>qepA</i>	0
Nalidixic acid	<i>gyrA</i> 83 (Ser-Leu)	44
Sulfamethoxazole-trimethoprim	<i>dfrA17</i>	30
	<i>dfrA1</i>	3
Tetracyclines	Only <i>tet</i> (A)	14
	Only <i>tet</i> (B)	28
	Only <i>tet</i> (C)	0
	<i>tet</i> (A) + <i>tet</i> (B)	8
	<i>tet</i> (B) + <i>tet</i> (C)	3
	<i>tet</i> (A) + <i>tet</i> (B) + <i>tet</i> (C)	2
	<i>tet</i> (D)	0
	<i>tet</i> (E)	0

shown in Table 3. A *bla*_{TEM} gene, identified by sequencing as *bla*_{TEM-1b}, was detected in 40 isolates (70.2%, 40/57). In 14 isolates (24.6%), *bla*_{SHV} was detected, and all of these genes were identified by sequencing as *bla*_{SHV-1}. The *bla*_{AmpC} genes were not detected in any of the isolates.

Overall, 83.3% (10/12) of the gentamicin-resistant isolates were PCR positive for the *aacC2* gene. The *aacC2* gene was detected in 10 isolates with PCR and confirmed based on DNA sequence. The results showed widespread presence of the *aacC2* gene among gentamicin-resistant uropathogenic *E. coli* isolates.

Of the 114 isolates tested, 28.9% (n=33) carried class 1 integrons. Although the gene cassettes present within each integron ranged in size from 1.0 to 2.0 kb, the most prevalent gene cassette size was around 1.7 kb. The 1.7 kb gene cassette contained the spectinomycin-resistant gene *aadA5* and the trimethoprim resistant gene *dfrA17*. No genes encoding resistance to tetracycline, chloramphenicol or ampicillin were found in the cassette arrays.

DISCUSSION

E. coli has been generally sensitive to antimicrobial agents, and this has made treatment simple [31]. First-line antibiotics are antibiotics that may be chosen empirically or based on culture and susceptibility results targeting a specific bacterium (*E. coli* in this case) with minimal im-

pact on other bacteria. The first-line antibiotics studied here included amoxicillin and ampicillin, which showed cross-resistance. Other generally effective antibiotics empirically used to treat first-time urinary tract infections are potentiated sulfonamides, cephalosporins, fluoroquinolones and chloramphenicols. They are bacteriostatic agents, which generally demonstrate good efficacy against Gram-positive and/or negative bacteria; however, more recently, several resistant strains have emerged, including strains resistant to aminopenicillins (ampicillin and amoxicillin), chloramphenicols (chloramphenicol and florfenicol), tetracyclines (oxytetracycline and doxycycline) and sulfamethoxazole-trimethoprim [15, 18]. Overusing the same type of antimicrobial agents, including prophylactic use to prevent surgical site infections or infections associated with other urogenital diseases, may have caused the emergence of resistant strains.

A high prevalence of oxytetracycline-resistant strains was observed in this study. The major mechanisms of tetracycline resistance are known to have efflux pump activity, ribosomal protection and enzymatic inactivation. Various *tet* genes confirm resistance via these mechanisms. Among the 40 tetracycline-resistant genes discovered to date, only the *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(E) genes were associated with the efflux mechanism [8, 43]. Most tetracycline-resistant genes have been found on mobile elements, either as plasmids or transposons [35]. The detection of *tet*(A), *tet*(B) and *tet*(C) genes in tetracycline-resistant uropathogenic *E. coli* isolates in pets indicated the primary mechanism to be active efflux. The presence of multiple tetracycline-resistance determinants was detected in 11 tetracycline-resistant strains (20%), which carried both *tet*(A) and *tet*(B) or both *tet*(B) and *tet*(C). Two strains (3.6%) harbored the genes for the *tet*(A), *tet*(B) and *tet*(C) genes. Although 13 of our isolates carried more than one *tet* gene, they did not exhibit higher MIC values. Bryan *et al.* explained this phenomenon with the theory that harboring more than one *tet* gene is due to strong selective pressures rather than a selective advantage [6]. In this study, there were 14 strains with a high level of resistance to oxytetracycline, but none of the 5 tetracycline resistance genes or combinations of them were found. One possibility might be the existence of an additional resistance gene, a potential efflux mechanism or other unidentified factors contributing to the resistance phenotype. The mechanism of resistance in these isolates is the subject of a further study.

Chloramphenicol and florfenicol are broad-spectrum antibiotics that have rarely been used in companion animals. Of the phenicols, only chloramphenicol was used infrequently at NTUVH and NCYUVTH. In this study, resistance to chloramphenicols was moderate, with 31.6% (36/114) of isolates resistant to chloramphenicol and florfenicol. Active efflux pumps (*cmlA* and *floR*) played important roles in intrinsic and acquired chloramphenicol and/or florfenicol resistances. Overexpression of efflux pumps affecting chloramphenicol or florfenicol has become increasingly common in *E. coli* [3]. However, the high incidence of florfenicol resistance in the *E. coli* isolates analyzed herein was somewhat unexpected. This finding suggested that the selective pressure of

chloramphenicol, as well as that the other antimicrobials, may have a relevant role in the emergence and dissemination of florfenicol resistance in *E. coli*. In addition, the *floR* gene was not detected in 18 isolates in which the MICs of florfenicol ranged from 8 to 256 mg/l. It may be that other mechanisms, such as overproduction of the AcrAB-TolC multidrug efflux system, may be responsible for florfenicol resistance in these isolates [38].

Multiple mechanisms were involved in the resistance to fluoroquinolones in *E. coli*. In addition to mutations in chromosomal genes encoding DNA gyrase and topoisomerase IV, a plasmid-mediated quinolone resistance (PMQR) mechanism has also been reported, including Qnr-mediated protection of DNA from quinolone binding, expression of a QepA-encoded efflux pump and *aac(6')-Ib-cr* mediated FQ acetylation [19, 36, 45, 48]. In the present study, *E. coli* urinary isolates from 6 dogs were resistant to enrofloxacin (MIC 32–128 mg/l). All enrofloxacin-resistant isolates had two point mutations, one in GyrA (Ser-83 to Leu) and one in ParC (Ser-80 to Ile) (Table 3). Each enrofloxacin/ciprofloxacin-resistant isolate in this study was also resistant to at least three other antibiotics that were commonly used to treat UTIs. Further characterization of the mechanisms of resistance to enrofloxacin/ciprofloxacin and to the other antibiotics assessed in this study is needed.

Our results demonstrate that canine uropathogenic *E. coli* isolates have a higher level of resistance to aminopenicillins (50.0% to ampicillin and 44.7% to amoxicillin) and a lower level of resistance to amoxicillin-clavulanic acid (2.6%). This result was consistent with beta-lactam antibiotics, mostly aminopenicillins, being the most commonly used class of antimicrobials in dogs. The results of the study indicated that *bla*_{TEM-1} was the most common β -lactamase among the ampicillin-resistant *E. coli* isolates (70.2%), in agreement with that results of previous studies that reported a high prevalence of the *bla*_{TEM-1b} gene among *E. coli* isolates [4, 46]. In addition, *bla*_{SHV-1} was found in 14 ampicillin-resistant isolates. The TEM-1 and SHV-1 enzymes were the predominant plasmid-mediated β -lactamases found in gram-negative Enterobacteria [5]. Thus, production of the TEM-1 and SHV-1 enzymes in uropathogenic canine *E. coli* is of concern. This study confirms the importance in β -lactamase detection in animals. The data indicate that the clinical usage of β -lactams in canine therapy imposes a strong selective pressure in the emergence of resistant bacterial isolates.

In addition to resistance to penicillins, fluoroquinolones, tetracyclines and chloramphenicols, the uropathogenic *E. coli* isolates were also resistant to other antimicrobials, such as spectinomycin and sulfonamide. Resistance to multiple antimicrobials, especially to spectinomycin and sulfonamides, was often associated with integrons, which are genetic elements that can acquire and spread antibiotic resistance genes [41]. Sulfonamides have been used alone or in combination with trimethoprim for the treatment of UTIs in humans. However, sulfonamides have been infrequently used in dogs, because of susceptibility to adverse effects [47]. Thus, according to our study, 28.9% (33/114) of the *E. coli* isolates causing UTIs possessed class I integrons, a

result that correlated with other reports [40]. However, many studies have documented a higher proportion of class I integrons among *E. coli* clinical isolates from farm animals: 52% of isolates from farm animals [11], 64% of swine diarrhea isolates [27] and 82% isolates from chickens [28]. Since *E. coli* strains from food production animals were more frequently exposed to antimicrobial pressure than those from companion animals [11], the differences between companion animals, such as cats and dogs, and farm animals may reflect differences in selective pressure due to antibiotic use [37].

Two veterinary hospitals in northern (NTUVH) and southern (NCYUVTH) Taiwan were selected for this study. The 2 hospitals were chosen for their similarities and dissimilarities important for a meaningful comparison of antimicrobial resistance of uropathogenic *E. coli* isolates. Both are university-affiliated teaching hospitals with innovative equipment and capable faculty/staff providing quality full inpatient and outpatient services. However, the geographical and demographic differences are distinct enough to show inequality in average incomes, animal breeds and density of private practices such that clinical case distributions and drug use could result in diversified antimicrobial resistances and yet be representative of Taiwan's current status.

Strong evidence supports an association between antibiotic use and resistance in hospitals [25, 30]. In this study, penicillin was the most often used antibiotic class in both animal hospitals, but the penicillins used were quite different. At NTUVH, older and cheaper penicillins with extended spectrum (amoxicillin and ampicillin) predominated, whereas the combination of amoxicillin and clavulanic acid, a commonly used antibiotic to treat sinusitis, bronchitis, otitis media, skin and tissue infections and UTIs, was most commonly used at NCYUVTH. This result suggested a direct link between the higher prevalence of resistance and continuous selective pressure by a single antibiotic. The resistance to sulfamethoxazole-trimethoprim, nalidixic acid and oxytetracycline among the most common pathogens causing urinary tract infections was higher at NTUVH than at NCYUVTH (Table 1). Gentamicin was also very commonly used at NCYUVTH due to its broad antimicrobial spectrum, low cost and convenient once-daily administration. The persistent use of gentamicin and other aminoglycosides may be related to the perception that they are essential components of therapy for life-threatening infections. Unfortunately, the possible toxicity of gentamicin is sometimes overlooked. The rate of resistance to enrofloxacin (13.3%) was also higher in NCYUVTH, as fluoroquinolones are now the most commonly prescribed therapy for UTIs [14, 23] and previous studies have shown cross-resistance to fluoroquinolones in *E. coli* isolated from UTIs [12, 13, 21]. The results of the present study indicated that all fluoroquinolone-resistant isolates (n=6) from NCYUVTH had higher MICs against enrofloxacin (≥ 32 mg/l) and that ciprofloxacin resistance (≥ 16 mg/l) was closely related to the number of topoisomerase mutations in GyrA (Ser-83 to Leu) and ParC (Ser-80 to Ile) (Tables 1 and 3).

The potential role of different infection control practices was not investigated in this study. However, this and a lon-

ger hospital stay, which increases the risk for colonization of resistant hospital clones from other small animal patients in the same environment, probably contributed to the higher resistance figures for NTUVH. Other factors that must be considered and are subject to further studies include the total antibiotic pressure in the community, adherence to guidelines for doses and duration of antibiotic treatment and the spread of resistant clones related to good infection control practices.

In conclusion, monitoring antibiotic usage and resistance patterns in a veterinary teaching hospital may serve as an early indicator of changes in the antibiotic susceptibility of clinical isolates. The combination of bacterial culturing and analysis of antibiotic susceptibility and resistance genes provided useful information for veterinarians in the management of persistent or recurrent UTI in dogs. Such extensive evaluation would be unnecessary in most less complicated UTIs in dogs, but the bacteria responsible for persistent infection often eventually incorporate nosocomial resistance plasmids and become increasingly difficult to manage. The techniques used in this study were straightforward and could be applied retrospectively, because the isolates had been maintained. Thus, retaining isolates early is advisable when persistent *E. coli* infection may become problematic, particularly when a dog has suspected impairments in resistance to colonization and when information about the pattern of *E. coli* strains is desired.

ACKNOWLEDGMENTS. The authors thank the National Taiwan University Veterinary Hospital and National Chiayi University Veterinary Teaching Hospital for generously providing the clinical *Escherichia coli* isolates.

REFERENCES

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**: 3389–3402. [Medline] [CrossRef]
- Ball, K. R., Rubin, J. E., Chirino-Trejo, M. and Dowling, P. M. 2008. Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002–2007. *Can. Vet. J.* **49**: 985–990. [Medline]
- Blickwede, M. and Schwarz, S. 2004. Molecular analysis of florfenicol-resistant *Escherichia coli* isolates from pigs. *J. Antimicrob. Chemother.* **53**: 58–64. [Medline] [CrossRef]
- Briñas, L., Moreno, M. A., Teshager, T., Sáenz, Y., Porrero, M. C., Domínguez, L. and Torres, C. 2005. Monitoring and characterization of extended-spectrum β -lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. *Antimicrob. Agents Chemother.* **49**: 1262–1264. [Medline] [CrossRef]
- Briñas, L., Zarazaga, M., Sáenz, Y., Ruiz-Larrea, F. and Torres, C. 2002. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. *Antimicrob. Agents Chemother.* **46**: 3156–3163. [Medline] [CrossRef]
- Bryan, A., Shapir, N. and Sadowsky, M. J. 2004. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. *Appl. Environ. Microbiol.* **70**: 2503–2507. [Medline] [CrossRef]
- Chen, Y. M., Wright, P. J., Lee, C. S. and Browning, G. F. 2003. Uropathogenic virulence factors in isolates of *Escherichia coli* from clinical cases of canine pyometra and feces of healthy bitches. *Vet. Microbiol.* **94**: 57–69. [Medline] [CrossRef]
- Chopra, I. and Roberts, M. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* **65**: 232–260. [Medline] [CrossRef]
- Clinical and Laboratory Standards Institute 2007. Performance Standards for Antimicrobial Susceptibility Testing. Seventeenth Informational (Supplement.): M100–S17 Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – 2nd ed. CLSI Document M31-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
- Cocchi, S., Grasselli, E., Gutacker, M., Benagli, C., Convert, M. and Piffaretti, J. C. 2007. Distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. *FEMS Immunol. Med. Microbiol.* **50**: 126–132. [Medline] [CrossRef]
- Cohn, L. A., Gary, A. T., Fales, W. H. and Madsen, R. W. 2003. Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. *J. Vet. Diagn. Invest.* **15**: 338–343. [Medline] [CrossRef]
- Cooke, C. L., Singer, R. S., Jang, S. S. and Hirsh, D. C. 2002. Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. *J. Am. Vet. Med. Assoc.* **220**: 190–192. [Medline] [CrossRef]
- Cotard, J. P., Gruet, P., Pechereau, D., Moreau, P., Pages, J. P., Thomas, E. and Deleforge, J. 1995. Comparative study of marbofloxacin and amoxicillin-clavulanic acid in the treatment of urinary tract infections in dogs. *J. Small Anim. Pract.* **36**: 349–353. [Medline] [CrossRef]
- Drazenovich, N., Ling, G. V. and Foley, J. 2004. Molecular investigation of *Escherichia coli* strains associated with apparently persistent urinary tract infection in dogs. *J. Vet. Intern. Med.* **18**: 301–306. [Medline] [CrossRef]
- Everett, M. J., Jin, Y. F., Ricci, V. and Piddock, L. J. 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. *Antimicrob. Agents Chemother.* **40**: 2380–2386. [Medline]
- Ewing, W.H. 1986. Edwards and Ewing's Identification of *Enterobacteriaceae* by Biochemical Reactions, 4th ed., Elsevier, New York.
- Féria, C. P., Correia, J. D., Machado, J., Vidal, R. and Gonçalves, J. 2000. Urinary tract infection in dogs. Analysis of 419 urocultures carried out in Portugal. *Adv. Exp. Med. Biol.* **485**: 301–304. [Medline] [CrossRef]
- Ferrero, L., Cameron, B., Manse, B., Lagneaux, D., Crouzet, J., Famechon, A. and Blanche, F. 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. *Mol. Microbiol.* **13**: 641–653. [Medline] [CrossRef]
- Forrester, S. D., Troy, G. C., Dalton, M. N., Huffman, J. W. and Holtzman, G. 1999. Retrospective evaluation of urinary tract infection in 42 dogs with hyperadrenocorticism or diabetes mellitus or both. *J. Vet. Intern. Med.* **13**: 557–560. [Medline] [CrossRef]

21. Gebru, E., Choi, M. J., Lee, S. J., Damte, D. and Park, S. C. 2011. Mutant prevention concentration and mechanism of resistance in clinical isolates and enrofloxacin/marbofloxacin selected mutants of *Escherichia coli* of canine origin. *J. Med. Microbiol.* **60**: 1512–1522. [Medline] [CrossRef]
22. Guardabassi, L., Loeber, M. E. and Jacobson, A. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Vet. Microbiol.* **98**: 23–27. [Medline] [CrossRef]
23. Hagman, R. and Greko, C. 2005. Antimicrobial resistance in *Escherichia coli* isolated from bitches with pyometra and from urine samples from other dogs. *Vet. Rec.* **157**: 193–196. [Medline]
24. Hagman, R. and Kühn, I. 2002. *Escherichia coli* strains isolated from the uterus and urinary bladder of bitches suffering from pyometra: comparison by restriction enzyme digestion and pulsed-field gel electrophoresis. *Vet. Microbiol.* **84**: 143–153. [Medline] [CrossRef]
25. Harbarth, S., Harris, A. D., Carmeli, Y. and Samore, M. H. 2001. Parallel analysis of individual and aggregated data on antibiotic exposure and resistance in gram-negative bacilli. *Clin. Infect. Dis.* **33**: 1462–1468. [Medline] [CrossRef]
26. Johnson, J. R., Kaster, N., Kuskowski, M. A. and Ling, G. V. 2003. Identification of urovirulence traits in *Escherichia coli* by comparison of urinary and rectal *E. coli* isolates from dogs with urinary tract infection. *J. Clin. Microbiol.* **41**: 337–345. [Medline] [CrossRef]
27. Kang, S. G., Lee, D. Y., Shin, S. J., Ahn, J. M. and Yoo, H. S. 2005. Changes in patterns of antimicrobial susceptibility and class 1 integron carriage among *Escherichia coli* isolates. *J. Vet. Sci.* **6**: 201–205. [Medline]
28. Keyes, K., Hudson, C., Maurer, J. J., Thayer, S., White, D. G. and Lee, M. D. 2000. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. *Antimicrob. Agents Chemother.* **44**: 421–424. [Medline] [CrossRef]
29. Ling, G. V., Norris, C. R., Franti, C. E., Eisele, P. H., Johnson, D. L., Ruby, A. L. and Jang, S. S. 2001. Interrelations of organism prevalence, specimen collection method, and host age, sex, and breed among 8,354 canine urinary tract infections (1969–1995). *J. Vet. Intern. Med.* **15**: 341–347. [Medline]
30. McGowan, J. E. Jr. 1983. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. *Rev. Infect. Dis.* **5**: 1033–1048. [Medline] [CrossRef]
31. Muratani, T. and Matsumoto, T. 2004. Bacterial resistance to antimicrobials in urinary isolates. *Int. J. Antimicrob. Agents* **24**: S28–S31. [Medline] [CrossRef]
32. Ng, L. K., Martin, I., Alfa, M. and Mulvey, M. 2001. Multiplex PCR for the detection of tetracycline resistant genes. *Mol. Cell. Probes* **15**: 209–215. [Medline] [CrossRef]
33. Normand, E. H., Gibson, N. R., Taylor, D. J., Carmichael, S. and Reid, S. W. 2000. Trends of antimicrobial resistance in bacterial isolates from a small animal referral hospital. *Vet. Rec.* **146**: 151–155. [Medline] [CrossRef]
34. Norris, C. R., Williams, B. J., Ling, G. V., Franti, C. E., Johnson, D. L. and Ruby, A. L. 2000. Recurrent and persistent urinary tract infections in dogs: 383 cases (1969–1995). *J. Am. Anim. Hosp. Assoc.* **36**: 484–492. [Medline] [CrossRef]
35. Roberts, M. C. 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol. Rev.* **19**: 1–24. [Medline] [CrossRef]
36. Robicsek, A., Strahilevitz, J., Jacoby, G. A., Macielag, M., Abbanat, D., Park, C. H., Bush, K. and Hooper, D. C. 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat. Med.* **12**: 83–88. [Medline] [CrossRef]
37. Rosser, S. J. and Young, H. K. 1999. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. *J. Antimicrob. Chemother.* **44**: 11–18. [Medline] [CrossRef]
38. Schwarz, S., Kehrenberg, C., Doublet, B. and Cloeckaert, A. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol. Rev.* **28**: 519–542. [Medline] [CrossRef]
39. Seguin, M. A., Vaden, S. L., Altier, C., Stone, E. and Levine, J. F. 2003. Persistent urinary tract infections and reinfections in 100 dogs (1989–1999). *J. Vet. Intern. Med.* **17**: 622–631. [Medline]
40. Shaheen, B. W., Oyarzabal, O. A. and Boothe, D. M. 2010. The role of class 1 and 2 integrons in mediating antimicrobial resistance among canine and feline clinical *E. coli* isolates from the US. *Vet. Microbiol.* **144**: 363–370. [Medline] [CrossRef]
41. Stokes, H. W. and Hall, R. M. 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. *Mol. Microbiol.* **3**: 1669–1683. [Medline] [CrossRef]
42. Sunde, M. 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. *J. Antimicrob. Chemother.* **56**: 1019–1024. [Medline] [CrossRef]
43. Thaker, M., Spanogiannopoulos, P. and Wright, G. 2010. The tetracycline resistome. *Cell. Mol. Life Sci.* **67**: 419–431. [Medline] [CrossRef]
44. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680. [Medline] [CrossRef]
45. Tran, J. H. and Jacoby, G. A. 2002. Mechanism of plasmid-mediated quinolone resistance. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 5638–5642. [Medline] [CrossRef]
46. Vo, A. T., van Duijkeren, E., Fluit, A. C. and Gastra, W. 2007. Characteristics of extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from horses. *Vet. Microbiol.* **124**: 248–255. [Medline] [CrossRef]
47. Williamson, N. L., Frank, L. A. and Hnilica, K. A. 2002. Effects of short-term trimethoprim-sulfamethoxazole administration on thyroid function in dogs. *J. Am. Vet. Med. Assoc.* **221**: 802–806. [Medline] [CrossRef]
48. Yamane, K., Wachino, J. I., Suzuki, S., Kimura, K., Shibata, N., Kato, H., Shibayama, K., Konda, T. and Arakawa, Y. 2007. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob. Agents Chemother.* **51**: 3354–3360. [Medline] [CrossRef]