J Antimicrob Chemother 2013; **68**: 1019–1024
doi:10.1093/jac/dks514 Advance Access publication 9 January 2013

Journal of Antimicrobial Chemotherapy

Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates recovered from companion animals in the USA

Bashar W. Shaheen[1], Rajesh Nayak[1]*, Steven L. Foley[1] and Dawn M. Boothe[2]

[1]*Division of Microbiology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, USA;*
[2]*Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA*

*Corresponding author. Tel: +1-870-543-7482; Fax: +1-870-543-7307; E-mail: rajesh.nayak@fda.hhs.gov

Received 8 September 2012; returned 12 October 2012; revised 9 December 2012; accepted 11 December 2012

Objectives: To determine the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants and investigate mutations in gyrase and topoisomerase genes that may contribute to increased fluoroquinolone resistance in canine and feline *Escherichia coli* isolates in the USA that displayed reduced susceptibility to extended-spectrum cephalosporins. This study was undertaken because previous epidemiological studies identified a potential correlation between extended-spectrum cephalosporins and fluoroquinolone resistance.

Methods: Isolates ($n=54$) with reduced susceptibility to ceftazidime or cefotaxime were screened by PCR for the presence of PMQR determinants and gyrase and topoisomerase genes were sequenced. Isolates were further characterized by conjugation and phylogenetic analyses.

Results: PMQR determinants *aac(6′)-Ib-cr*, *qnrS* and *qepA* were identified in 30, 23 and 5 isolates, respectively. Multiple mutations were identified in the quinolone resistance-determining region, including the novel substitutions of Glu-84 → Ala and Leu-88 → Gln in ParC and Arg-432 → Ser and Glu-460 → Val in ParE. The isolate that exhibited the highest level of enrofloxacin resistance (MIC >256 mg/L) had a double mutation in *gyrA* (Ser-83 → Leu and Asp-87 → Asn) and a triple mutation in *parC* (Ser-80 → Ile, Glu-84 → Gly and a novel mutation, Leu-88 → Gln). The presence of PMQR genes increased the ciprofloxacin MIC values 4-fold to 8-fold in transconjugants relative to the recipient strain. Approximately 39% of the isolates belonged to phylogenetic group D and 30% to group B2, which typically contain an increased number of virulence determinants compared with other groups.

Conclusions: Novel mutations in topoisomerase genes and PMQR determinants *aac(6′)-Ib-cr*, *qnrS* and *qepA* genes were detected among extended-spectrum β-lactamase-producing *E. coli* in the USA.

Keywords: *qnr*, *qepA*, *aac(6′)-Ib-cr*, topoisomerase IV, ESBLs

Introduction

Escherichia coli are commonly isolated from clinical specimens from dogs and cats, as a cause of urinary tract infection and pyometra.[1] Several fluoroquinolones (FQs) have been approved for treatment of canine and feline clinical infections.[2] Consequently, increasing resistance to FQs in *E. coli* has been observed worldwide,[3] thereby limiting the therapeutic options or resulting in treatment failure. Treatment can be further complicated when FQ-resistant *E. coli* isolates exhibit multidrug resistance phenotypes.[2,4] Resistance to quinolones is primarily attributed to chromosomal mutations in DNA gyrase and/or topoisomerase IV genes or mutations in the regulatory genes of the *acrAB* efflux pump.[5,6] Changes in cell membrane permeability and overexpression of the multidrug efflux pump are complementary mechanisms that contribute to high-level FQ resistance in clinical isolates.[4,5,7] In addition, plasmid-mediated quinolone resistance (PMQR) genes, such as the target protection PMQR gene *qnr*, the enzymatic modification gene *aac(6′)-Ib-cr* and the efflux pump gene *qepA*, have also been shown to reduce the susceptibility to quinolones.[6]

Several epidemiological surveys have been conducted to identify the presence of PMQR in *E. coli* strains from humans and food animals.[6,8,9] However, limited studies describe the prevalence and the role of PMQR determinants and novel mutations, such as those found in *gyrA*, *parC* and *parE*, in mediating resistance to FQs in *E. coli* isolates from companion animals in the USA.[5,6,10] Studies have also reported an epidemiological link

between PMQR genes and those encoding extended-spectrum β-lactamases (ESBLs) or other β-lactamases in *E. coli* isolates of human origin.[6,8–10] Therefore, we have determined the prevalence of the PMQR determinants *qnr*, *aac(6′)-Ib-cr* and *qepA*, and investigated mutations in gyrase and topoisomerase genes that may contribute to increased FQ resistance in canine and feline *E. coli* isolates that displayed reduced susceptibility to extended-spectrum cephalosporins (ESCs).[11]

Materials and methods

Bacterial isolates and susceptibility testing

Susceptibility testing was performed on 944 canine and feline clinical *E. coli* isolates collected from clinical veterinary laboratories between May 2008 and May 2009.[11] The antimicrobial MICs for all isolates were determined at the Clinical Pharmacology Laboratory at Auburn University using custom microdilution susceptibility plates. The MIC results were interpreted according to the CLSI interpretive standards.[12,13] *E. coli* isolates ($n=54$) that exhibited reduced susceptibility to ceftazidime or cefotaxime (MIC $\geq$16 mg/L) were selected for the study.[11] These bacterial strains were isolated from urine ($n=38$), skin wound ($n=5$) and soft tissue ($n=11$) specimens.

Detection of resistance genes and DNA sequence analysis

All 54 *E. coli* isolates were screened for PMQR genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr* or *qepA*] and β-lactamase genes (bla_{TEM}, bla_{CMY-2} and $bla_{CTX-M-1}$ group) by PCR using specific primers and conditions (Table S1, available as Supplementary data at *JAC* Online). The *qnr*-positive, *aac(6′)-Ib-cr*-positive and *qepA*-positive control strains, *E. coli* J53 pMG252 (*qnrA1*), J53 pMG298 [*qnrB1* and *aac(6′)-Ib-cr*], J53 pMG306 (*qnrS1*), J53 p2007057 (*qnrD*) and J53 pAT851 (*qepA*) and *Proteus mirabilis* 06-498 (*qnrC*), were provided by Dr George Jacoby (Lahey Clinic Medical Center, Burlington, MA, USA). All *E. coli* isolates that tested positive for *aac(6′)-Ib-cr* were confirmed by enzymatic digestion with BtsCI restriction endonuclease (New England Biolabs, Ipswich, MA, USA) or by DNA sequencing. The PCR products for *qepA* and *qnrS* genes were sequenced. PCR amplification and DNA sequencing of the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC* and *parE* were performed using primers listed in Table S1.

Conjugation and plasmid analyses

Conjugation experiments were performed on seven *E. coli* (donor) isolates to determine the transferability and the contribution of PMQR-carrying plasmids in mediating resistance to FQs. Conjugation assays were performed using plate mating experiments with selected donors, including representatives of *aac(6′)-Ib-cr*-, *qnrS*- and *qepA*-positive isolates and an azide-resistant recipient strain of *E. coli* (*E. coli* J53 AZ^r) as previously described.[14] All transconjugants were tested for susceptibility to the following antimicrobials using standard veterinary microdilution plates (CMV1AGNF, Trek Diagnostics, Cleveland, OH, USA): amikacin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline and trimethoprim/sulfamethoxazole. Donors and transconjugants were analysed by S1 nuclease-PFGE and Southern blot analysis as previously described.[11]

Phylogenetic grouping

E. coli isolates were examined for their phylogenetic groups A, B1, B2 and D based on triplex PCR and the presence or absence of *chuA*, *yjaA* and *tspE4.C2* genes.[15]

Results

Mutations in topoisomerase genes

Point mutations in the quinolone resistance-determining regions of *gyrA*, *parC* and *parE*, involving an amino acid substitution, were detected in 49 (91%) of the 54 *E. coli* isolates exhibiting reduced susceptibility to ESCs (Table 1). No mutations were identified in *gyrB* genes. In four isolates, novel substitutions Glu-84→Ala (NCTR-914) and Leu-88→Gln (NCTR-915) in *parC* and Glu-460→Val (NCTR-930) and Arg-432→Ser (NCTR-888) in *parE* topoisomerase IV genes were identified.

PMQR determinants

Thirty-eight (70%) of 54 phenotypic ESBL-producing *E. coli* isolates were positive for PMQR determinants, with *aac(6′)-Ib-cr*-type, *qnrS*-type or *qepA*-type alleles detected in 30 (56%), 23 (43%) and 5 (9%) *E. coli* isolates, respectively (Table 1). The *qnrA*, *qnrB*, *qnrC* and *qnrD* genes were not detected in our study.

PMQR determinants and association with ESBL genotype

Among *E. coli* that produce ESBLs, $bla_{CTX-M-15}$ was prominent and detected in 78% (42/54) of isolates (Table 1). A $bla_{CTX-M-14}$ group gene was detected in 11 of 23 *qnrS*-positive isolates; 8 were $bla_{CTX-M-24}$ and 3 were $bla_{CTX-M-14}$. Moreover, TEM-1, TEM-33 and CMY-2-type β-lactamases were detected in 15, 2 and 20 of *qnrS*-positive isolates, respectively. The $bla_{CTX-M-24}$, $bla_{CTX-M-14}$, bla_{CMY-2}, bla_{TEM-1} and bla_{TEM-33} genes were detected in 11, 4, 26 and 21, respectively, and in 3 of 30 *aac(6′)-Ib-cr*-positive isolates. Of the five *qepA*-positive isolates, $bla_{CTX-M-24}$, $bla_{CTX-M-14}$, bla_{CMY-2} and bla_{TEM-1} genes were detected in four, one, five and four isolates, respectively.

Phylogenetic analysis

Nearly 69% (37/54) of *E. coli* isolates belonged to phylogenetic group D (21/54) or B2 (16/54), while the remaining isolates belonged to group A (9/54) or group B1 (8/54) (Table 1).

Conjugation experiments

Plasmids were transferred by conjugation from all seven isolates used as donors. The ciprofloxacin MICs for seven transconjugants harbouring PMQR *aac(6′)-Ib-cr* or *qepA* ranged from 0.06 to 0.12 mg/L, representing an increase of 4-fold to 8-fold compared with the recipient, *E. coli* J53 AZ^r (Table 2). Southern blotting of S1-PFGE products revealed plasmids ranging from 33 to 160 kb and probes for the *aac(6′)-Ib-cr* gene were hybridized with plasmids from donor strains and their respective transconjugants ($n=7$; Figure S1, available as Supplementary data at *JAC* Online).

Table 1. Antimicrobial susceptibility testing and resistance genes and phylogenetic data for *E. coli* isolated from canines and felines

Isolate (NCTR) number	Tissue source	Species	*gyrA*	*parC*	*parE*	CTX-M-1 group	CTX-M-14 group	CMY	TEM	*qnrS*	*aac(6')-Ib-cr*	*qepA*	Phylogenetic group	Enrofloxacin MIC (mg/L)
934	urine	canine	–	–	–	ND	–	–	–	–	+	–	B2	0.06
938	urine	feline	–	–	–	ND	–	–	–	+	+	–	B2	0.06
941	urine	canine	–	–	–	ND	–	CMY-2	–	+	+	–	B2	0.125
936	urine	canine	–	–	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	–	B2	0.25
928	vagina	canine	–	–	–	CTX-M-15	CTX-M-14	CMY-2	TEM-1	–	–	–	B2	0.5
888	urine	canine	G81D	–	R432S	CTX-M-15	–	–	–	+	–	–	B1	1
929	urine	canine	S83L	–	–	ND	CTX-M-14	CMY-2	TEM-1	–	+	+	B2	1
940	urine	feline	S83L	–	–	CTX-M-15	CTX-M-14	CMY-2	TEM-1	+	+	–	B2	2
899	urine	canine	S83L, D87Y	S80I	–	ND	–	CMY-2	TEM-1	+	–	–	D	16
889	urine	canine	S83L, D87N	S80I	–	CTX-M-15	CTX-M-24	CMY-2	–	+	–	+	B1	16
923	urine	canine	S83L, D87N	S80I, E84V	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	–	B2	32
890	urine	feline	S83L, D87N	S80I, E84V	–	CTX-M-15	CTX-M-14	CMY-2	TEM-33	+	+	–	B2	32
891	urine	canine	S83L, D87N	S80I	–	CTX-M-15	–	CMY-2	TEM-1	+	+	–	A	32
917	urine	feline	S83L, D87N	S80I,E84G	–	CTX-M-15	CTX-M-24	CMY-2	TEM-33	+	+	–	D	32
916	ear	canine	S83L, D87N	S80I, E84G	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	+	D	32
905	urine	canine	S83L, D87N	S80I, E84V	–	CTX-M-15	CTX-M-24	–	TEM-1	–	–	–	B2	64
907	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-14	CMY-2	TEM-1	–	–	–	B2	64
919	urine	canine	S83L, D87N	S80I	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	–	–	A	64
933	nasal structure	canine	S83L, D87N	S80I	–	ND	CTX-M-14	CMY-2	–	–	–	–	D	64
893	abdomen	canine	S83L, D87N	S80I	S458A	ND	–	CMY-2	TEM-1	+	–	–	A	64
906	urine	canine	S83L, D87N	S80I	S458A	ND	CTX-M-24	CMY-2	TEM-1	+	–	–	A	64
903	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	TEM-1	–	+	–	D	64
908	sinus	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	TEM-1	–	+	–	D	64
909	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	–	–	–	+	–	D	64
918	wound	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	TEM-33	–	+	–	D	64
924	urine	canine	S83L, D87N	S80I, E84V	–	CTX-M-15	–	CMY-2	TEM-1	–	+	–	B2	64
935	urine	canine	S83L, D87N	S80I	S458A	ND	–	CMY-2	–	–	+	–	A	64
892	wound	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	TEM-1	+	+	–	A	64
894	wound	canine	S83L, D87N	S80I, E84G	–	CTX-M-15	–	CMY-2	TEM-1	+	+	–	D	64
895	ear	canine	S83L, D87N	S80I, E84G	S458T	CTX-M-15	–	CMY-2	–	+	+	–	A	64
900	anal sac	canine	S83L, D87N	S80I	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	+	+	–	D	64
901	urine	canine	S83L, D87N	S80I, E84G	–	CTX-M-15	–	CMY-2	TEM-1	+	+	–	D	64
902	nasal structure	feline	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	TEM-1	+	+	–	D	64
930	urine	canine	S83L, D87N	S80I	E460V	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	+	B2	64
904	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	TEM-1	+	+	+	D	64
910	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	TEM-1	–	–	–	D	128
920	urine	feline	S83L, D87N	S80I, E84G	–	CTX-M-15	CTX-M-24	CMY-2	TEM-181	–	–	–	B1	128
925	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	–	–	–	–	D	128
937	urine	canine	S83L, D87N	S801, E84V	–	CTX-M-15	CTX-M-14	CMY-2	–	–	–	–	B2	128
896	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	TEM-1	+	–	–	B2	128
898	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	–	+	–	–	B1	128

Continued

Table 1. *Continued*

Isolate (NCTR) number	Tissue source	Species	*gyrA*	*parC*	*parE*	CTX-M-1 group	CTX-M-14 group	CMY	TEM	*qnrS*	*aac(6′)-Ib-cr*	*qepA*	Phylogenetic group	Enrofloxacin MIC (mg/L)
912	wound	feline	S83L, D87N	S80I	S458T	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	–	A	128
931	anal sac	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	+	–	D	128
897	urine	canine	S83L, D87N	S80I	S458A	ND	–	CMY-2	TEM-1	+	+	–	D	128
911	wound	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	–	TEM-1	+	+	–	A	128
913	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	–	CMY-2	–	–	–	–	D	256
914	urine	canine	S83L, D87N	S80I, E84A	–	CTX-M-15	–	CMY-2	TEM-30	–	–	–	B1	256
921	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-14	CMY-2	TEM-1	–	–	–	D	256
922	vulva	canine	S83L, D87N	S80I, E84G	–	CTX-M-15	CTX-M-24	CMY-2	TEM-1	–	–	–	B1	256
926	trachea	canine	S83L, D87N	S80I, E84G	–	ND	CTX-M-24	CMY-2	TEM-181	–	–	–	B1	256
927	urine	canine	S83L, D87N	S80I, E84G	–	ND	CTX-M-14	CMY-2	TEM-181	–	–	–	B1	256
939	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-14	CMY-2	TEM-1	+	–	–	D	256
932	urine	canine	S83L, D87N	S80I	S458A	CTX-M-15	CTX-M-14	CMY-2	TEM-1	–	+	–	D	256
915	urine	canine	S83L, D87N	S80I, E84G, L88Q	–	CTX-M-15	CTX-M-24	CMY-2	–	–	–	–	B2	>256

ND, not determined.

Discussion

Nearly 6% (54/944) of isolates selected for this study exhibited reduced susceptibility to ESCs. Of these, 85% (46/54) were also resistant to FQs, thus ∼5% (46/944) of *E. coli* strains isolated from the companion animals exhibited co-resistance to FQ and ESCs. Few studies have investigated the prevalence of PMQR determinants in *E. coli* from companion animals.[10,16] In our study, PMQR determinants *aac(6′)-Ib-cr* (56%) and *qnrS* (43%) were most prevalent, followed by *qepA* (9%), in the *E. coli* isolates exhibiting reduced susceptibility to ESCs. The *qepA* gene, which codes for an efflux pump, was first identified from *E. coli* strains of human origin in Japan.[17] To the best of our knowledge, the current study is the first report of the prevalence of *qepA* gene among *E. coli* strains from companion animals in the USA. The detection of *aac(6′)-Ib-cr* and *qepA* has been reported elsewhere in *E. coli* from dogs[10] and pigs.[18] These PMQR determinants contributed only minimally to FQ resistance in isolates devoid of mutations in the quinolone resistance-determining region of *gyrA* (Table 1). However, previous studies have indicated that PMQR determinants in strains can facilitate the selection of higher-level FQ resistance compared with plasmid-free strains.[6] Additionally, several studies have indicated a high prevalence of PMQR determinants among ESBL-producing Enterobacteriaceae.[6,8–10] Our study showed that PMQR determinants can co-exist with $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{CMY} alleles, which are the most prevalent types in the USA.[11]

Previous studies have shown that the primary target of FQ in *E. coli* is DNA gyrase and mutations in the gyrase genes can lead to development of decreased susceptibility to FQs, thus complicating the treatment of infections.[4,5] Typically, a mutation in *gyrA* leading to the Ser-83 → Leu substitution is the first *gyrA* mutation observed, which leads to a reduction in susceptibility; however, high-level FQ resistance (ciprofloxacin MIC >4 mg/L) requires an additional mutation in *gyrA* at position 87 and at least one *parC* mutation at either position 80 or 84.[19,20] In the present study, no isolates with a single mutation in *parC* at Ser-80 → Ile without the concurrent *gyrA* double mutation at Ser-83 → Leu and Asp-87 → Asn were detected (Table 1). Hence, the present study could not directly characterize the impact of just the concurrent *gyrA* mutations at Ser-83 → Leu and Asp-87 → Asn without the *parC* mutation. However, the presence of all three mutations in the isolates was associated with high-level enrofloxacin resistance (≥16 mg/L; Table 1), which correlates with the results of other studies.[5,19,20] In addition, the high percentages of groups D and B2, which generally contain more virulence factors,[15] found among *E. coli* isolates from companion animals potentially reflect a higher level of virulence in these isolates.

In summary, the PMQR genes, which are located on conjugative plasmids, conferred reduced susceptibility to ciprofloxacin relative to the recipient *E. coli* strain. Mutations in the gyrase and topoisomerase IV genes play a greater role in mediating increased FQ resistance in *E. coli* than PMQR determinants. The emergence and dissemination of conjugative PMQR determinants in canine and feline *E. coli* isolates can serve as a potential reservoir of multidrug resistance genes. Because FQs and ESCs remain some of the more effective treatment options in companion animals,[2,4,11] it is of concern that their clinical use may drive the dissemination of PMQR determinants in *E. coli* isolates

Table 2. MICs, antimicrobial resistance genes and plasmid profiles of *E. coli* isolates used in conjugation experiments

	MIC (mg/L) of antimicrobial agent															Presence or absence of						Plasmids	
Group and isolate	AMK	AMC	AMP	FOX	TIO	CRO	CHL	CIP	GEN	KAN	NAL	STR	FIS	TET	SXT	bla_{TEM}	bla_{CMY}	$bla_{CTX\text{-}M\text{-}1}$ group	*qepA*	*aac(6′)-Ib-cr*	*qnrS*	plasmid size (kb)	antimicrobials used for selection
Clinical isolates (donors)																							
NCTR 895	8	32	>32	8	>8	>64	16	>4	1	32	>32	>64	>256	>32	>4	−	+	+	−	+	+	160, 94	—
NCTR 931	8	>32	>32	>32	>8	>64	8	>4	1	32	>32	≤32	>256	>32	≤0.12	+	+	+	−	+	−	116, 55	—
NCTR 930	>64	>32	>32	>32	>8	64	8	>4	>16	>64	>32	>64	>256	>32	>4	+	+	+	+	+	−	90	—
NCTR 892	16	32	>32	16	>8	>64	8	>4	2	32	>32	≤32	>256	>32	≤0.12	+	+	+	−	+	+	113, 59	—
NCTR 904	>64	32	>32	>32	>8	>64	8	>4	>16	>64	>32	>64	>256	>32	>4	+	+	+	+	+	+	94, 82	—
NCTR 923	16	>32	>32	32	>8	>64	8	>4	1	>64	>32	64	>256	>32	≤0.12	+	+	+	−	+	−	95, 33	—
NCTR 924	8	>32	>32	8	>8	>64	8	>4	1	64	>32	≤32	256	>32	≤0.12	+	+	+	−	+	−	98, 39	—
Recipient, J53 AZ^r	1	8	4	≤4	0.5	≤0.25	8	≤0.015	1	≤8	4	≤32	≤16	≤4	≤0.12	−	−	−	−	−	−	ND	
Transconjugants																							
Trans 895	4	32	>32	4	>8	64	8	0.06	≤0.25	16	4	≤32	>256	>32	>4	−	−	+	−	+	−	160	TET
Trans 931	4	>32	>32	>32	>8	>64	8	0.12	0.5	16	4	≤32	32	>32	≤0.12	+	+	+	−	+	−	116, 55	TET
Trans 930	>64	32	>32	8	2	0.5	8	0.12	>16	>64	4	≤32	>256	≤4	>4	+	−	+	+	+	−	90	AMP
Trans 892	4	16	>32	8	>8	64	8	0.12	0.5	32	8	≤32	32	>32	≤0.12	+	+	+	−	+	−	139	AMP
Trans 904	>64	32	>32	8	1	0.5	8	0.06	>16	>64	4	≤32	>256	≤4	>4	+	−	+	+	+	−	82	GEN
Trans 923	2	32	>32	8	>8	64	8	0.06	≤0.25	32	4	≤32	≤16	32	≤0.12	+	−	+	−	+	−	119, 33	AMP
Trans 924	8	32	>32	8	>8	>64	4	0.12	1	16	4	≤32	32	32	≤0.12	+	+	+	−	+	−	128, 37	AMP

AMK, amikacin; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; FOX, cefoxitin; TIO, ceftiofur; CRO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; ND, not determined.

from companion animals. Judicious use of FQs and ESCs by veterinarians is warranted to preserve the efficacy of these drug classes in treating diseases in dogs and cats.

Acknowledgements

We thank Dr George A. Jacoby at Lahey Clinic for kindly providing *qnr*-positive control strains and the *E. coli* J53 AZ^r strain. We thank Idexx Laboratories and the Morris-Animal Foundation for their collaborations with D. M. B. We also thank Allen Gies of the University of Arkansas for Medical Sciences Core Sequencing Facility for DNA sequencing. We are grateful to Drs John B. Sutherland, Mark Hart, Fatemeh Rafii and Carl E. Cerniglia for their critical review of the manuscript before submission. We thank Dr Fatemeh Rafii and Miseon Park for their technical assistance with Southern blotting and Dr Carl E. Cerniglia for his encouragement and support of this work.

Funding

This work was supported by US FDA funds. B. W. S. is supported by the Oak Ridge Institute for Science and Education.

Transparency declarations

None to declare.

Disclaimer

The views presented in this manuscript do not necessarily reflect those of the US FDA.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Hagman R, Kuhn I. *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* 2002; **84**: 143–53.

2 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**: 190–2.

3 Shaheen BW, Boothe DM, Oyarzabal OA *et al*. Antimicrobial resistance profiles and clonal relatedness of canine and feline *Escherichia coli* pathogens expressing multidrug resistance in the United States. *J Vet Intern Med* 2010; **24**: 323–30.

4 Shaheen BW, Boothe DM, Oyarzabal OA *et al*. Evaluation of the contribution of *gyrA* mutation and efflux pumps to fluoroquinolone and multidrug resistance in pathogenic *Escherichia coli* isolates from dogs and cats. *Am J Vet Res* 2011; **72**: 25–32.

5 Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents* 2005; **25**: 358–73.

6 Strahilevitz J, Jacoby GA, Hooper DC *et al*. Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 2009; **22**: 664–89.

7 Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* 2006; **19**: 382–402.

8 Robicsek A, Strahilevitz J, Jacoby GA *et al*. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; **12**: 83–8.

9 Robicsek A, Strahilevitz J, Sahm DF *et al*. *qnr* prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother* 2006; **50**: 2872–4.

10 Ma J, Zeng Z, Chen Z *et al*. High prevalence of plasmid-mediated quinolone resistance determinants *qnr*, *aac(6′)-Ib-cr*, and *qepA* among ceftiofur-resistant Enterobacteriaceae isolates from companion and food-producing animals. *Antimicrob Agents Chemother* 2009; **53**: 519–24.

11 Shaheen BW, Nayak R, Foley SL *et al*. Molecular characterization of resistance to extended-spectrum cephalosporins in clinical *Escherichia coli* isolates from companion animals in the United States. *Antimicrob Agents Chemother* 2011; **55**: 5666–75.

12 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals: Approved Standard M31-A3*. CLSI, Wayne, PA, USA, 2008.

13 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

14 Wang M, Tran JH, Jacoby GA *et al*. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob Agents Chemother* 2003; **47**: 2242–8.

15 Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555–8.

16 Pomba C, da Fonseca JD, Baptista BC *et al*. Detection of the pandemic O25-ST131 human virulent *Escherichia coli* CTX-M-15-producing clone harboring the *qnrB2* and *aac(6′)-Ib-cr* genes in a dog. *Antimicrob Agents Chemother* 2009; **53**: 327–8.

17 Yamane K, Wachino J, Suzuki S *et al*. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrob Agents Chemother* 2008; **52**: 1564–6.

18 Liu JH, Deng YT, Zeng ZL *et al*. Coprevalence of plasmid-mediated quinolone resistance determinants QepA, Qnr, and AAC(6′)-Ib-cr among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. *Antimicrob Agents Chemother* 2008; **52**: 2992–3.

19 Bagel S, Hullen V, Wiedemann B *et al*. Impact of *gyrA* and *parC* mutations on quinolone resistance, doubling time, and supercoiling degree of *Escherichia coli*. *Antimicrob Agents Chemother* 1999; **43**: 868–75.

20 Heisig P. Genetic evidence for a role of *parC* mutations in development of high-level fluoroquinolone resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 1996; **40**: 879–85.