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ORIGINAL PAPER

In vitro potency and efficacy favor later generation fluoroquinolones for treatment of canine and feline *Escherichia coli* uropathogens in the United States

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Abstract Information regarding in vitro activity of newer fluoroquinolones (FQs) is limited despite increasing resistance in canine or feline pathogenic *Escherichia coli* (*E. coli*). This study describes in vitro potency and efficacy toward *E. coli* of seven FQs grouped according to similarities in chemical structure: enrofloxacin, ciprofloxacin, orbifloxacin (first-group), levofloxacin, marbofloxacin (second-group) and pradofloxacin, moxifloxacin (third-group; latest S, S-pyrrolidino-piperidine at C-7). Potency measures included minimum inhibitory concentration (MIC) (geometric mean MIC, MIC_{50}, MIC_{90}); and mutant prevention concentration (MPC) for FQ susceptible isolates only. In vitro efficacy measures included relative susceptibility ($MIC_{BP\text{-}S}$:MIC) or resistance (MIC:$MIC_{BP\text{-}R}$) and mutant selection window (MSW) (MPC:MIC). For enrofloxacin susceptible isolates, mean MIC (μg/ml) was least for each third-group drug and ciprofloxacin and greatest for enrofloxacin and orbifloxacin ($P = 0.006$). For enrofloxacin susceptible isolates, MPC were below MIC:$MIC_{BP\text{-}R}$ and least for pradofloxacin (0.29 ± 0.16 μg/ml) and greatest for enrofloxacin (1.55 ± 0.55 μg/ml) ($P = 0.006$). MSW was least for pradofloxacin (55 ± 30) and greatest for ciprofloxacin (152 ± 76) ($P = 0.0024$). $MIC_{BP\text{-}S}$:MIC was greatest ($P = 0.025$) for pradofloxacin (190.1 ± 0.61) and least for enrofloxacin (23.53 ± 0.83). For FQ susceptible isolates, FQs MIC:$MIC_{BP\text{-}R}$ may serve as a surrogate for MPC. Because in vitro efficacy was greatest for pradofloxacin; it might be preferred for treatment of urinary tract infections (UTIs) associated with FQ susceptible *E. coli* uropathogens.

Keywords *Escherichia coli* · MIC · MPC · Fluoroquinolone · Canine · Feline

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Introduction

Escherichia coli are a common cause of canine and feline urinary tract infections (UTIs) and fluoroquinolones (FQs) antimicrobial compounds are commonly used to treat UTIs in dogs and cats (Liu et al. 2012). The FQs commonly are categorized into different "generations" based on chemical structure and chronological approval. Chemical modifications, including the addition of fluorine to the progenitor of all FQs, broadened the antimicrobial spectrum, enhanced pharmacokinetics (e.g., absorption and tissue distribution) (Martinez et al. 2006), and particularly for newer generation drugs, decreased in vitro resistance (Scoper 2008).

Since approval of the first veterinary FQ in the late 1980's (Hopkins et al. 2005; Martinez et al. 2006), trends of increasing resistance to FQs in *E. coli* from companion animals have been reported worldwide (Cooke et al. 2002; Hopkins et al. 2005; Shaheen et al. 2011). In one study, approximately 20–40 % of *E. coli*, collected from canine or feline *E. coli* pathogens of various veterinary teaching hospitals in the USA were resistance to FQs (Boothe et al. 2006). Moreover, the type of resistance expressed by *E. coli* also is changing, with more isolates expressing multidrug resistance (MDR) in the Europe, Canada and USA (Authier et al. 2006; Cohn et al. 2003; Normand et al. 2000; Shaheen et al. 2011).

FQs resistance limits therapeutic options for treatment of infections. Among the strategies by which the incidence of resistance might be reduced is therapeutic use of drugs which are either more effective (more potent toward the target organism) or are more able to avoid resistance. Modifications in the chemical structure of FQs has increased the spectrum and potency and appears to have decreased resistance (Ball 2000). Data comparing the activity of newer versus earlier FQs among differing resistant phenotypes (including MDR) of companion animal *E. coli* isolates is limited (Wetzstein 2005). The extent of *E. coli* cross-resistance among the differing generations of FQs also is not clear: susceptibility testing of canine or feline uropathogenic *E. coli* frequently finds an isolate to be susceptible to one FQ and resistant to another. The purpose of this study was to describe and compare the in vitro potency and efficacy of seven different FQs against canine and feline uropathogenic *E. coli* isolates, with a focus on assessing whether or not later generation FQs might offer a clinical advantage for treatment of canine or feline *E. coli.*

Materials and methods

Bacterial strains

Representative isolates ($n = 161$, including 122 canine and 39 feline isolates) for each phenotype was randomly selected from a working subpopulation of isolates. This working subpopulation had been selected to represent, based on MIC distribution and resistance phenotypes, a surveillance study population of 1,500, which were acquired between May 2008 and June 2010. Isolates had been cultured from canine or feline urine samples of animals suspected of UTIs and submitted to veterinary diagnostic laboratory (IDEXX Reference Laboratories, Inc.) for identification and confirmation. Upon receipt in our laboratory, each *E. coli* isolate was re-cultured on BBL CHROMagar® *E. coli* agar plates (CHROMagar, Paris, France) at 37 °C for 18–24 h to confirm isolate identification as *E. coli*, and then the isolates were stored in Trypticase soy broth (Difco, MD) containing 30 % glycerol at −80 °C. Antimicrobial susceptibility testing to fourteen drugs: ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, cefpodoxime, cefotaxime, ceftazidime, meropenem, enrofloxacin, gentamicin, doxycycline, chloromphenicol, cefotaxime/clavulanic acid and trimethoprim/sulfamethoxazole was performed using custom microdilution susceptibility plates according to the manufacturer's protocol (Trek Diagnostic Systems, Cleveland, OH). Six drug classes were represented by these drugs, including the β-lactams (penicillins and cephalosporoins), tetracyclines, chloramphenicol, FQs, aminoglycosides and folic acid inhibitors. The isolates for this study ($n = 161$) were categorized into the following resistance phenotypes: no drug resistance (NDR; $n = 50$), resistant to a single drug or drug class (SDR, resistance was expressed only to β-lactams; $n = 50$) or resistant to two or more classes of antibacterial agents, i.e., multiple (MDR; $n = 61$). MDR phenotypes included isolates both susceptible to enrofloxacin (ENR^S-MDR; $n = 30$) and resistance to enrofloxacin (ENR^R-MDR; $n = 31$). Enrofloxacin-susceptible isolates included all NDR and SDR isolates as well as ENR^S-MDR isolates. Isolates were classified into four geographical regions based on origin: South (North Carolina), West (California), Midwest (Ohio and Illinois), and Northeast (Massachusetts).

Antimicrobial drugs

Isolates were tested against the following drugs: enrofloxacin, ciprofloxacin, marbofloxacin, levofloxacin, and orbifloxacin (Sigma Aldrich, St. Louis, MO), pradofloxacin (Bayer, KS, USA), and moxifloxacin (Alcon laboratories, Inc, TX). The selection of the FQs was based on their use for treatment of various canine or feline infections. Enrofloxacin, marbofloxacin and orbifloxacin have been approved for use exclusively in dogs and cats in USA, while pradofloxacin has been approved in Europe recently, and under investigation in USA. Three remaining drugs are approved for use in humans but also were used in USA by veterinarians: ciprofloxacin, levofloxacin, and moxifloxacin. Chemical purity of all drugs was ≥98.0 %. Stock solutions of the drugs were prepared daily and stored in −20 °C until use.

In general, FQs used for the treatment of bacterial infections in humans or animals have been categorized as first through fourth generation drugs. However, the criterion for categorization of each drug is not consistent, and includes both chemical structural differences as well as timing of approval. The lack of discrete criteria for each generation complicates assignment. Accordingly, for our study, we grouped the FQs based on similarities in chemical structure: first-group being those drugs with or without a or a halogen at C-8 but with a piperidine at C-7(enrofloxacin, its demethylated metabolite ciprofloxacin and orbifloxacin), second-group being those with a piperidine at C-7, and with a 1,8-cyclo structure (levofloxacin and marbofloxacin) and third-group being those drugs with *S*, *S*-pyrrolidino-piperidine at C-7, and with an N or methoxy at C-8 (moxifloxacin and pradofloxcin) (Fig. 1). This categorization is comparable to other investigators in regards to grouping of the drugs based on chemical structure(Martinez et al. 2006).

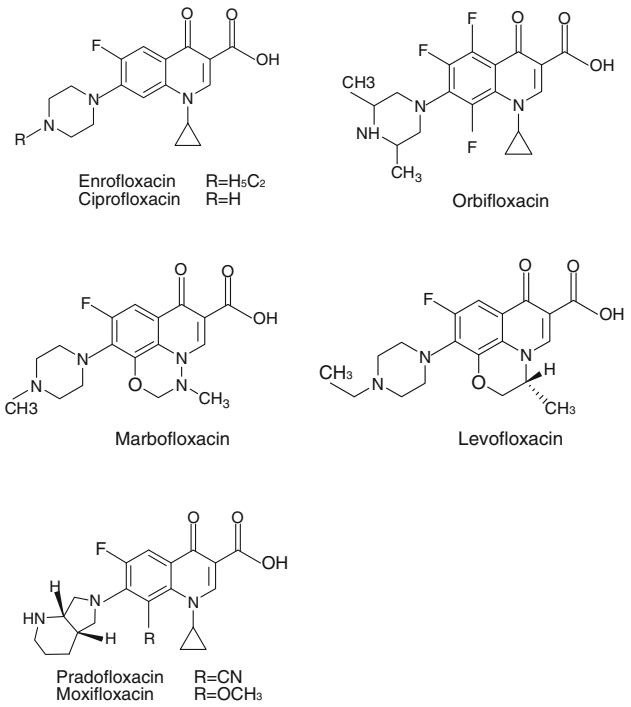

Fig. 1 Chemical structures of seven FQs studied

Minimal inhibitory concentration (MIC) determinations

In vitro susceptibility tests of each of the seven studied FQs were performed by the broth microdilution method using Muller-Hinton broth (Difco, MD), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2008, 2012) for measuring the MIC. Start cultures were streaked on Trypticase soy agar plates (Difco, MD) and incubated at 37 °C for 18–24 h. One clone was harvested and suspended in 4.5 ml of 0.9 % normal saline and adjusted to 0.5 McFarland standard turbidity ($\sim 10^8$ c.f.u.) using the SENSITITER® Nephelometer (TREK Diagnostic Systems, Cleveland, OH) before testing. All MIC determinations were performed in triplicate and reference strain *E. coli* ATCC® 25922 (American Type Culture Collection, Manassas, VA) was included as a measure of intra-and inter-assay quality control in all tests. The MIC values were recorded by use of the SENSITITER® VIZION system (TREK Diagnostic Systems, Cleveland, OH). Interpretative criteria were those recommended by CLSI (Institute 2008) for enrofloxacin, ciprofloxacin, marbofloxacin, levofloxacin, orbifloxacin; Since no CLSI breakpoints and interpretive criteria are available for pradofloxacin and moxifloxacin, their respective breakpoints were those recommended by European Medicine Agency (EMEA) (EMEA/CVMP 2007) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST 2011). For pradofloxacin, the breakpoints are comparable to the peak plasma drug concentration achieved in the plasma of drugs at the approved dose (Boothe 2006). The breakpoints for FQs are listed in Table 1 along with the range of concentrations used for MIC determination for each drug.

Mutant prevention concentration (MPC) determinations

The MPCs experiments were determined as described previously (Marcusson et al. 2005; Pasquali and Manfreda 2007) with minor modifications. MPC were performed on FQ-susceptible isolates randomly chosen among the differing phenotypes (NDR = 3, SDR = 3, and ENR^S-MDR = 3) based on the MIC determinations, and *E. coli* ATCC® 25922 as a reference strain. These strains were fully susceptible to FQs. Briefly, start cultures were inoculated on Trypticase soy agar plates and incubated at 37 °C for 18–24 h. One colony was selected and grown in Luria–Bertani broth (Difco, MD) at 37 °C overnight again, and then 100 µl cultures were transferred into 100 ml of Muller-Hinton broth for the incubation at 37 °C with shaking for about 2.5–3 h until an OD_{540} of ~ 1.0 was reached. Aliquots of 10 ml of culture were centrifuged at $4{,}000 \times g$ for 15 min, and then resuspended in the remaining liquid. Aliquots of 200 µl, containing $\geq 10^{10}$ c.f.u./ml, were spread on Muller-Hinton agar plates containing increasing concentration of seven FQs at concentrations equal to 1–256 × MIC, respectively. Plates were incubated at 37 °C for a total of 96 h and examined every 24 h for the appearance of colonies. MPC was recorded as the lowest antibiotic concentration at which no colonies grew on an agar plate. For each strain, MPC was determined in duplicate to ensure reproducibility. The frequencies of mutation were also determined as the number of colonies appearing on the plate with antibiotic divided by the number of colonies in the inoculums.

Outcome measures and statistical analysis

For each drug and group of FQ, outcome measures were determined for all isolates and then for each phenotype. However, because all isolates resistant to enrofloxacin expressed MDR, we reported MDR isolates as either ENR^S-MDR or ENR^R-MDR. We also reported statistics for all enrofloxacin susceptible isolates, including all NDR, SDR and ENR^S-MDR isolates (Table 2).

Measures of potency reported included mean MIC (determined as geometric mean, MIC_{50}, MIC_{90}) and MPC; mean ± standard deviation were reported unless data was not normally distributed; in such instances, median and range were reported. Several outcome measures were determined to represent potential clinical efficacy. For each drug, each isolate was designated as either resistant or not (binary response) and the proportion of susceptible versus

Table 1 The breakpoint of seven FQs used in this study

Isolate(s)	MIC (μg/ml) of						
	ENR	CIP	ORB	MAR	LEV	PRA	MOX
CLSI susceptible breakpoints	≤0.5	≤1	≤1	≤1	≤2	≤1[a]	≤0.5[b]
CLSI resistant breakpoints	≥4	≥4	≥8	≥4	≥8	≥2[a]	>1[b]
ATCC® 25922 MIC range	0.008–0.03	0.004–0.015	0.004–0.015	0.008–0.03	0.008–0.063	0.004–0.015	0.008–0.063

[a] Breakpoints from European Medicine Agency (EMA)

[b] Breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

resistant isolates were determined for each drug. To assess how susceptible each isolate was to each drug, we defined relative susceptibility as the ratio of the susceptible MIC_{BP} to MIC in enrofloxacin susceptible isolates ($MIC_{BP\text{-}S}$:MIC). The further the isolate MIC was from the $MIC_{BP\text{-}S}$ (that is, the larger the $MIC_{BP\text{-}S}$:MIC ratio), the more likely effective concentrations will be achieved at the site of infection and thus the more susceptible the isolate presumably is in vitro to the drug of interest. In addition to relative susceptibility, we also determined relative resistance (the ratio of MIC to resistant breakpoint [$MIC_{BP\text{-}R}$]). Finally, for each enrofloxacin susceptible isolate, the mutant selection window (MSW) was determined based on the ratio of MPC to MIC.

Bonferroni's test of multiple comparisons was used to identify statistical differences in the proportion of resistant isolates among the drugs tested. Comparisons among drugs were made using the analysis of variance (ANOVA) procedure for potency and efficacy outcome measures, including geometric mean, MIC_{50} and MIC_{90} and the ratios defining relative susceptibility and resistance and MSW; for ratios, the log ratio was the basis for comparison. Statistics were performed using SAS 9.1 statistical software (SAS Institute Inc., NC, US). Values of $P \leq 0.05$ were considered significant.

Results

Comparison of potency among drugs

The in vitro MIC statistics are delineated for all isolates and by phenotype in Table 2. Among the drugs, for all isolates, pradofloxacin was the most and enrofloxacin and orbifloxacin the least potent ($P = 0.007$). For enrofloxacin susceptible isolates, mean MIC (μg/ml) ($P = 0.007$) and MIC_{90} ($P = 0.032$) also were least for pradofloxacin and greatest for enrofloxacin and orbifloxacin ($P = 0.007$) (Table 2). When grouped according to generation, the MIC ranges for all isolates were 0.002–256 μg/ml, 0.004–64 μg/ml and 0.002–32 μg/ml for the first-group (earliest generation), second and third-group (latest generation), respectively, while the median MIC were 0.099, 0.038, and 0.028 μg/ml, respectively. The highest concentration at which all isolates were inhibited were, for third-group, 8 (pradofloxacin), and 32 μg/ml (moxifloxacin), second-group (levofloxacin and marbofloxacin) at 32–64 μg/ml, and for the first-group (ciprofloxacin, enrofloxacin and orbifloxacin) at 64–256 μg/ml (Table 2). Among ENR^R-MDR isolates, the MIC_{90} of pradofloxacin, marbofloxoacin, levofloxacin, and moxifloxacin (8–32 μg/ml) were at least 4 times ($P = 0.01$) lower than those of enrofloxacin and orbifloxacin (128–256 μg/ml).

The MPC for enrofloxacin susceptible isolates are delineated for each drug in Table 3. The mean MPC (μg/ml) order of the seven FQs was pradofloxacin (0.29 ± 0.16) moxifloxacin < levofloxacin < marbofloxacin, ciprofloxacin < orbifloxacin < enrofloxacin (1.55 ± 0.55) ($P = 0.006$). The lowest MPC were recorded for pradofloxacin (0.063–0.5 μg/ml) regardless of the phenotype with MPC being approximately two to five times lower than those for other FQs. The ranges of MPC for the third, second, and first-group were 0.063–1 μg/ml, 0.25–2 μg/ml and 0.25–2 μg/ml, respectively. A significant difference could not be detected for MPC among groups. The mutation frequencies of seven FQs for ten *E. coli* isolates were also determined in this study. Different FQs had varied mutation frequencies for *E. coli*. The mutation frequencies order of seven FQs was pradofloxacin (1.9×10^{-7} to $<1 \times 10^{-10}$) < moxifloxacin (4×10^{-7} to $<1 \times 10^{-10}$) < levofloxacin (1.2×10^{-6} to $<1 \times 10^{-10}$) < marbofloxacin (3×10^{-6} to $<1 \times 10^{-10}$) < cipofloxacin (1.4×10^{-5} to $<2 \times 10^{-10}$) < enrofloxacin (4×10^{-5} to $<6 \times 10^{-10}$), orbifloxacin (6.4×10^{-5} to $<9 \times 10^{-10}$). In all cases, the frequency decreased with increasing drug concentration on the selection plates.

Comparison of potential in vitro efficacy among drugs

Neither a numerical nor a statistical difference could be detected in the percent of isolates resistant to any drug. Further, most isolates (98.76 %) expressing resistance to one FQ expressed resistance to all FQs (cross-resistance).

For enrofloxacin-susceptible isolates, based on mean ratios, this study demonstrates that relative susceptibility was greatest ($P = 0.025$) for pradofloxacin (190.1 ± 0.61), followed by levofloxacin, ciprofloxacin, and marbofloxacin,

Table 2 Population statistics of minimum inhibitory concentrations (MICs) for uropathogenic *E. coli* isolates to seven FQs

	Type	ENR	CIP	ORB	MAR	LEV	PRA	MOX
MIC (μg/ml) range	NDR	0.004–0.125	0.002–0.03	0.008–0.125	0.004–0.03	0.004–0.063	0.002–0.015	0.004–0.125
	SDR	0.004–0.125	0.002–0.03	0.008–0.125	0.004–0.125	0.004–0.03	0.002–0.015	0.004–0.063
	ENR^S-MDR	0.004–0.063	0.004–0.25	0.015–1	0.004–0.5	0.004–0.25	0.002–0.125	0.002–0.063
	All ENR^S	0.004–0.125	0.002–0.25	0.008–1	0.004–0.5	0.004–0.25	0.002–0.125	0.004–0.125
	ENR^R-MDR	4–256	0.25–64	2–256	0.5–64	0.25–32	0.015–8	0.25–32
	All Isolates	0.004–256	0.002–64	0.008–256	0.004–64	0.004–32	0.002–8	0.002–32
MIC_{Mean*}	NDR	0.023	0.011	0.044	0.009	0.009	0.004	0.008
	SDR	0.022	0.009	0.027	0.011	0.009	0.005	0.01
	ENR^S-MDR	0.017	0.013	0.044	0.013	0.012	0.007	0.011
	All ENR^s	0.021	0.011	0.036	0.011	0.011	0.005	0.011
	ENR^R-MDR	35.79	10.00	53.52	10.69	7.48	3.42	7.65
	All isolates	0.088	0.064	0.147	0.04	0.035	0.019	0.037
MIC_{50}	NDR	0.03	0.008	0.063	0.008	0.008	0.004	0.008
	SDR	0.03	0.008	0.03	0.008	0.008	0.004	0.008
	ENR^S-MDR	0.015	0.008	0.03	0.012	0.012	0.008	0.015
	All ENR^s	0.03	0.015	0.03	0.008	0.008	0.004	0.008
	ENR^R-MDR	64	16	64	16	8	4	8
	All Isolates	0.03	0.015	0.03	0.015	0.008	0.004	0.015
MIC_{90}	NDR	0.063	0.03	0.125	0.015	0.015	0.008	0.03
	SDR	0.063	0.015	0.063	0.017	0.015	0.008	0.015
	ENR^S-MDR	0.063	0.015	0.069	0.03	0.03	0.015	0.03
	All ENR^s	0.063	0.03	0.063	0.015	0.015	0.008	0.015
	ENR^R-MDR	128	32	256	32	16	8	32
	All Isolates	64	16	64	16	8	4	8
Mean $MIC_{BP\text{-}S}$/MIC	All ENR^S	23.53 ± 0.83	115.96 ± 0.69	27.43 ± 0.67	93.21 ± 0.66	185.82 ± 0.72	190.1 ± 0.61	46.02 ± 0.48
Mean MIC/$MIC_{BP\text{-}R}$	All ENR^R	8.95 ± 1.43	2.50 ± 1.37	6.35 ± 1.29	2.68 ± 1.24	0.94 ± 1.21	1.71 ± 0.44	3.83 ± 0.93

ENR enrofloxacin, *CIP* ciprofloxacin, *ORB* orbifloxacin (first group), *MAR* marbfloxacin and *LEV* levofloxacin (second group), *PRA* pradofloxacin and *MOX* moxifloxacin (third group), *NDR* no drug resistance (n = 50), *SDR* single drug resistance (n = 50), *MDR* multiple drug resistance (n = 61) which included ENR^S enrofloxacin susceptible isolates, n = 30 and ENR^R enrofloxacin resistant isolates, n = 31, $MIC_{BP\text{-}S}$ susceptible breakpoints, $MIC_{BP\text{-}R}$ resistant breakpoints

*Mean was determined as geometric mean

Table 3 Seven FQs MICs and MPCs for FQ susceptible uropathogenic *E. coli* isolates

Strains ID	MIC (μg/ml)							MPC (μg/ml)						
	ENR	CIP	ORB	MAR	LEV	PRA	MOX	ENR	CIP	ORB	MAR	LEV	PRA	MOX
ACTT® 25922	0.004	0.002	0.015	0.004	0.004	0.002	0.008	0.5	0.25	1	0.25	0.5	0.063	0.5
Ec1[a]	0.03	0.008	0.03	0.008	0.015	0.004	0.008	2	1	2	1	1	0.25	1
Ec2[a]	0.015	0.008	0.008	0.015	0.015	0.004	0.008	1	1	0.5	2	1	0.5	0.5
Ec3[a]	0.03	0.015	0.03	0.008	0.008	0.004	0.004	2	2	1	1	1	0.125	0.5
Ec4[b]	0.03	0.008	0.03	0.015	0.015	0.015	0.015	2	0.5	2	0.5	1	0.25	1
Ec5[b]	0.03	0.008	0.015	0.03	0.008	0.008	0.015	1	2	2	1	0.5	0.25	1
Ec6[b]	0.008	0.004	0.015	0.015	0.008	0.004	0.008	1	0.25	0.5	1	1	0.25	0.5
Ec7[c]	0.008	0.008	0.03	0.015	0.015	0.008	0.015	2	1	2	1	1	0.5	1
Ec8[c]	0.015	0.004	0.03	0.008	0.015	0.008	0.004	2	0.5	2	1	1	0.25	0.25
Ec9[c]	0.063	0.004	0.03	0.008	0.004	0.008	0.008	2	1	1	0.5	0.5	0.5	0.5
Mean	0.023	0.007	0.023	0.013	0.011	0.007	0.009	1.55	0.95	1.40	0.93	0.85	0.29	0.68
Std	0.017	0.004	0.009	0.007	0.005	0.004	0.004	0.55	0.63	0.66	0.47	0.24	0.16	0.29
Median	0.022	0.008	0.03	0.011	0.011	0.006	0.008	2	1	1.5	1	1	0.25	0.5

Strains ID	MSW (MPC/MIC)						
	ENR	CIP	ORB	MAR	LEV	PRA	MOX
ACTT® 25922	125	125	67	63	125	31	63
Ec1[a]	67	125	67	125	67	63	125
Ec2[a]	67	125	63	133	67	125	63
Ec3[a]	67	267	33	125	125	31	125
Ec4[b]	67	63	67	17	67	17	67
Ec5[b]	33	250	133	33	125	31	67
Ec6[b]	125	63	33	67	125	63	63
Ec7[c]	250	125	67	67	67	63	67
Ec8[c]	133	125	67	125	67	63	63
Ec9[c]	32	250	33	63	125	63	63
Mean	97	152	63	82	96	55	76
Std	65	76	29	42	31	30	26
Median	67	125	67	67	96	63	65

[a] NDR = 3

[b] SDR = 3

[c] ENR^S-MDR = 3, and ATCC® 25922

and least for orbifloxacin and enrofloxacin (23.53 ± 0.83) (Table 2). For resistant isolates (ENR^R-MDR) isolates, relative resistance was greatest toward enrofloxacin (8.95 ± 1.43) and least for levofloxacin (0.94 ± 1.21) and pradofloxacin (1.71 ± 0.44) ($P = 0.043$) (Table 2). In terms of the relationship between MPC and MIC, the MSW was lowest for pradofloxacin (median 55) and greatest for ciprofloxacin (152) ($P = 0.0024$) (Table 3).

Discussion

This study describes in detail the microbiological activity of seven FQs, each of which is currently used in veterinary medicine, toward United States canine and feline uropathogenic *E. coli* that vary in their resistance phenotypes. Our descriptors included measures of potency (MIC and MPC) as well as in vitro efficacy (based on the ratio of $MIC/MIC_{BP\text{-}S}$, $MIC/MIC_{BP\text{-}R}$, and MSW).

Based on the population statistics in this study, MIC were equal to or even lower than those previously reported by our laboratory 10 years ago for five FQs (excluding pradofloxacin) toward susceptible *E. coli* isolates collected at a veterinary teaching hospital (Boothe et al. 2006). As such, our studies suggest that FQs remain highly potent toward susceptible canine and feline *E. coli* uropathogens, and pradofloxacin shows more effective antimicrobial activity, including some enrofloxacin susceptible isolates.

One reason is that the chemical modifications of pradofloxacin and other later generation drugs render them more potent toward susceptible organisms (Ball 2000; Scoper 2008; Wetzstein 2005).

Among the limitations of MIC is its inhibitory endpoint. During the last two decades, the MPC has been promoted in lieu of the MIC for designing dosing regimens (Drlica and Schmitz 2002; Liang et al. 2011; Sindelar et al. 2000). Targeting the MIC is likely to facilitate the emergence of the subpopulation mutants whereas targeting the MPC decreases the risk of their emergence as the predominant population. As such, designing doses such that concentrations at the site of infection exceed the MPC has been suggested as a method by which the selection of mutants during antibiotic treatment could be minimized (Blondeau et al. 2001; Liang et al. 2011). The lowest MPC was recorded in this study was for pradofloxacin; mean MPC was two to five times lower than that of enrofloxacin. Our mean MPC for pradofloxacin is similar to that determined in a previous study of feline or canine *E. coli* European isolates collected in 2004 ($n = 10$; $MPC_{90} = 0.175–2.0$ μg/ml). Our study suggests that pradofloxacin has the greatest potential reaching the MPC and thus killing concentrations for infecting *E. coli* inocula. It further demonstrated that FQ with the C-8 methoxy (such as pradofloxacin) compared to those with no substitution are characterized by lower MPC (Ince and Hooper 2001; Kowalski et al. 2003; Wetzstein 2005).

As measures of potency, neither MIC nor MPC offer insight into relative activity among the FQs. In order to compare this, we "normalized" MIC measures to CLSI breakpoints (EMEA breakpoints for pradofloxacin and EUCAST breakpoints for moxifloxacin), CLSI has recently updated recommendations for susceptibility testing such that it has adopted selected EUCAST interpretation strategies (Hombach et al. 2012), thus assessing either relative susceptibility or relative resistance. Based on the distance between MIC and the susceptible MIC breakpoint, our study demonstrates that pradofloxacin was characterized by the greatest relative susceptibility. This, coupled with its smallest MSW, suggests that among the FQs we studied, it has the greatest potential efficacy toward susceptible *E. coli* uropathogens. The distinction of susceptible isolates is important because this study also demonstrates variable levels of cross-resistance to nearly all FQs tested except pradofloxacin. As such, later generation FQs offer no therapeutic advantages to earlier generation drugs once FQ resistance has emerged in clinical *E. coli* isolates. However, this study does suggest that beginning treatment of FQ naïve *E. coli* with a higher level FQ may be prudent. That a higher generation drug might be more prudent also is supported by the poor performance of enrofloxacin in this study; it was characterized by the least potency, and was second only to ciprofloxacin in terms of magnitude of MSW. In vivo, enrofloxacin is de-ethylated to ciprofloxacin such that up to 50 % of bioactivity can result from this active metabolite (Boothe et al. 2002). However, because CLSI has not adjusted the breakpoints for enrofloxacin based on this active metabolite, this study did not take that into account. It is likely that enrofloxacin might perform better in terms of relative susceptibility (but not MSW since ciprofloxacin has the widest MSW of the drugs studied here).

In summary, the data from this study indicate that resistance to one FQ can result in resistance to nearly all FQs except pradofloxacin and that, for susceptible isolates, the resistant MIC breakpoint of each FQ may be a reasonable surrogate target for MPC when designing dosing regimens. Further, the later generation drug pradofloxacin is characterized by greater in vitro potency and potential efficacy toward susceptible canine or feline uropathogenic *E. coli*.

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Conflict of interest The authors declare that they have no conflict of interest.

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