

Commonality among Fluoroquinolone-Resistant Sequence Type ST131 Extraintestinal *Escherichia coli* Isolates from Humans and Companion Animals in Australia^{∇†}

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Escherichia coli sequence type 131 (ST131), an emergent multidrug-resistant extraintestinal pathogen, has spread epidemically among humans and was recently isolated from companion animals. To assess for human-companion animal commonality among ST131 isolates, 214 fluoroquinolone-resistant extraintestinal *E. coli* isolates (205 from humans, 9 from companion animals) from diagnostic laboratories in Australia, provisionally identified as ST131 by PCR, selectively underwent PCR-based O typing and *bla*_{CTX-M-15} detection. A subset then underwent multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) analysis, extended virulence genotyping, antimicrobial susceptibility testing, and fluoroquinolone resistance genotyping. All isolates were O25b positive, except for two O16 isolates and one O157 isolate, which (along with six O25b-positive isolates) were confirmed by MLST to be ST131. Only 12% of isolates (25 human, 1 canine) exhibited *bla*_{CTX-M-15}. PFGE analysis of 20 randomly selected human and all 9 companion animal isolates showed multiple instances of ≥94% profile similarity across host species; 12 isolates (6 human, 6 companion animal) represented pulsotype 968, the most prevalent ST131 pulsotype in North America (representing 23% of a large ST131 reference collection). Virulence gene and antimicrobial resistance profiles differed minimally, without host species specificity. The analyzed ST131 isolates also exhibited a conserved, host species-independent pattern of chromosomal fluoroquinolone resistance mutations. However, eight (89%) companion animal isolates, versus two (10%) human isolates, possessed the plasmid-borne *qnrB* gene ($P < 0.001$). This extensive across-species strain commonality, plus the similarities between Australian and non-Australian ST131 isolates, suggest that ST131 isolates are exchanged between humans and companion animals both within Australia and intercontinentally.

Escherichia coli is a major cause of extraintestinal infection in both humans and companion animals (2, 42). Such isolates exhibit resistance to multiple antimicrobial classes, including fluoroquinolones, with increasing frequency worldwide, thus posing a significant treatment challenge to physicians and veterinarians alike.

The virulent *E. coli* clonal group sequence type 131 (ST131), which is associated with fluoroquinolone resistance and belongs to phylogenetic group B2, has shown recent and rapid global dissemination. This clonal group is an emerging human pathogen in many parts of the world, including continental Europe (3, 33), the United Kingdom (28), Turkey (52), Korea (30), Canada (36, 38), and the United States (18, 19). Its association with *bla*_{CTX-M-15}, which encodes the CTX-M-15 extended-spectrum β-lactamase (ESBL), has also influenced

the recent pandemic spread of extended-spectrum *E. coli* cephalosporin resistance (3, 4, 18, 28, 29, 34, 44).

Recently, ST131 has also been isolated from sporadic cases of extraintestinal infection in companion animals (13, 22, 24, 40). This supports previous evidence that extraintestinal pathogenic *E. coli* (ExPEC) from humans and companion animals, particularly strains belonging to group B2, share similar virulence genotypes and phylogenetic origins (25, 31).

In a previous study by our group, fluoroquinolone-resistant (FQ^r) *E. coli* from extraintestinal infections in humans and companion animals were collected over a 2-year period in Eastern Australia (39). Clonal group ST131 accounted for 35% of human isolates and 7% of companion animal isolates (39). Here, we sought to further characterize these ST131 isolates for a broad range of molecular and phenotypic traits, both to assess for clonal commonality across host species within Australia and to compare these isolates to a broader international collection.

MATERIALS AND METHODS

Bacterial isolates. As reported elsewhere (39), a collection of 707 FQ^r clinical extraintestinal *E. coli* isolates from humans ($n = 582$) and companion animals ($n = 125$) from Eastern Australia (October 2007 to October 2008) underwent PCR-based determination of major *E. coli* phylogenetic group (A, B1, B2, and

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TABLE 1. Primers used to detect plasmid-mediated fluoroquinolone resistance genes

Target gene(s)	Primer	Sequence (5'-3') ^a	Product size (bp)	Source or reference
<i>aac(6')-Ib</i> and <i>aac(6')-Ib-cr</i>	aac6 for aac6 rev	ATGACTGAGCATGACCTTGC TTAGGCATCACTGCGTGTTTC	519	This study This study
<i>qnrA</i>	qnrA for qnrA rev	TCAGCAAGAGGATTTCTCA GGCAGCACTATGACTCCCA	626	31 31
<i>qnrB</i>	qnrB for qnrB rev	GGCGAAAAAATTTACAGAAA TCYGAATTGGTCARATCRCA	525	This study This study
<i>qnrS</i>	qnrS for qnrS rev	TGCAAGTTTCCAACAATGCC GATCTAAACCGTCGAGTTCCG	367	This study This study
<i>qepA</i>	qepA for qepA rev	CTTCTGCCCCGAGTATCGTG GCAGGTCCAGCAGCGGGTAG	217	51 51

^a IUPAC code: R = A or G; Y = C or T.

D) (5). The 263 group B2 isolates (i.e., 250 human and 13 companion animal [12 canine, 1 feline] isolates) were screened for ST131 status by PCR-based detection of ST131-specific single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* (23). The 214 (205 human, 9 companion animal) thus-identified ST131 isolates (39), which due to the timing and geography of the cases suggested no link among the animal cases, or between animal versus human cases, underwent further characterization here.

Initial characterization of isolates. The 214 putative ST131 isolates were screened by PCR for the ST131-associated O25b *rfb* variant (7) and *bla*_{CTX-M-15} (6). Isolates that were not type O25b ($n = 3$) underwent PCR for 12 additional sepsis-associated *rfb* variants (O1, O2, O4, O6, O7, O12, O15, O16, O18, O25a, O75, and O157) (6). For confirmation of the ST131 status, nine presumptive ST131 isolates, of which six (three human and three canine ST131 isolates) exhibited the O25b *rfb* variant and three (all human) exhibited unique non-O25b *rfb* variants, underwent confirmatory multilocus sequence typing (MLST) according to the Achtman system (<http://mlst.ucc.ie/mlst/dbs/Ecoli>), using DNA sequence analysis of seven housekeeping genes (50). Approximately 10% of human ST131 isolates (20/205; 9.8%) were randomly selected for comparison with all nine companion animal ST131 isolates (eight canine, one feline) according to multiple additional characteristics, determined as described below.

PFGGE. Genomic DNA of the 29 selected ST131 isolates underwent XbaI pulsed-field gel electrophoresis (PFGE) according to a standardized protocol (41). Dice coefficient-based similarity dendrograms were constructed within BioNumerics (Bio-Rad, Applied Maths) according to the unweighted pair group method with arithmetic means. Isolates were considered to represent the same pulsotype if they exhibited $\geq 94\%$ profile similarity, which corresponds with a ≤ 3 -band overall difference (45). PFGE profiles were compared both within this collection and to an unpublished PFGE reference library comprising 533 *E. coli* ST131 isolates, obtained from around the world, but mainly the United States and Canada (23; J. R. Johnson, unpublished data [see also Table 2]).

Virulence genotyping. The 29 selected ST131 isolates were screened by PCR for 53 ExPEC-associated virulence genes and variants (17, 20, 26, 35, 47). Virulence scores were the numbers of virulence genes detected for each isolate. The virulence genotype similarity between two isolates was calculated as the number of shared virulence genes divided by the combined total number of virulence genes.

Susceptibility testing. All isolates underwent disk diffusion susceptibility testing to enrofloxacin (9), with fluoroquinolone resistance defined as nonsusceptibility. The 29 selected ST131 isolates underwent disk diffusion susceptibility testing to 18 additional antimicrobial agents (amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefoxitin, ceftazidime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, nitrofurantoin, piperacillin, streptomycin, piperacillin-tazobactam, tetracycline, and trimethoprim-sulfamethoxazole) and determination of broth microdilution MICs for four fluoroquinolones (enrofloxacin, ciprofloxacin, moxifloxacin, and pradofloxacin, a new veterinary fluoroquinolone) (48). The methods and interpretive criteria used were as specified by the Clinical and Laboratory Standards Institute (8, 9), except for pradofloxacin (resistance breakpoint, 2 $\mu\text{g}/\text{ml}$) (14).

Detection of chromosomal fluoroquinolone resistance mutations. The quinolone resistance-determining region (QRDR) of *gyrA*, encoding subunit A of DNA gyrase (primary fluoroquinolone target), was screened for resistance mu-

tations by a rapid pyrosequencing-based method using the primers *gyrA*-5'-+211 (5'-GGTGACGTAATCGGTAAATATCA-3') and *gyrA*-3'-+329-biotin (5'-CCGAAGTTACCCTGACCATCTA-3'). The cycling conditions were as follows: 5 min of denaturation at 94°C; 35 cycles of 94°C for 30s, 61°C for 40 s, and 72°C for 45 s; and then 5 min of elongation at 72°C. The resulting 118-bp PCR fragment was analyzed by using the sequencing primer *gyrA*-5'-+222-EcoMPC-seq (5'-CGGTAATACCATCCCA-3') on a PSQ MA96 Pyrosequencer according to the manufacturer's instructions (Biotage, Uppsala, Sweden). The corresponding region of *parC*, encoding subunit A of topoisomerase IV (secondary fluoroquinolone target), was amplified and sequenced as described previously (16). SNPs were defined relative to the wild-type *E. coli* strain (WT).

Detection of plasmid-mediated resistance genes. The plasmid-mediated quinolone resistance genes *qnrA*, *qnrB*, *qnrS*, and *qepA*, which encode three target-protecting proteins and an efflux pump, respectively, were screened for by multiplexing existing and novel single PCRs, with a second PCR screen used to identify isolates containing *aac(6')-Ib*. The primers and product sizes are listed in Table 1; controls were as described previously (11). PCR conditions were: 5 min of denaturation at 95°C; 30 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 50 s; and then 10 min of elongation at 72°C. All *aac(6')-Ib*-positive isolates underwent digestion of the PCR product with FokI and NdeI (New England Biolabs, Ipswich, MA) to identify the *aac(6')-Ib-cr* variant, which encodes a bifunctional aminoglycoside-fluoroquinolone modifying enzyme (27).

Efflux pump activity testing. Two methods were used to detect efflux pump activity, including MIC testing in the presence of an efflux pump inhibitor and an organic solvent tolerance test. For efflux pump inhibitor testing, broth microdilution susceptibility to the four studied fluoroquinolones was determined in duplicate in the presence or absence of 64 μg of L-phenylalanyl-arginyl- β -naphthylamide (PA β N; Sigma P 4157)/ml (8, 9). For organic solvent tolerance, overnight cultures (ca. $\sim 10^9$ CFU/ml) of each isolate underwent four 10-fold serial dilutions. Approximately 1- μl portions of the last three dilutions (ca. 10^5 , 10^6 , and 10^7 CFU/ml) were spotted onto the surface of an LB agar plate and allowed to dry. Then, 15 ml of an organic solvent (hexane, cyclohexane, and hexane-cyclohexane combinations of 3:1, 1:1, and 1:3) was overlaid on the plate to a depth of 2 to 3 mm. The plates were sealed with petroleum jelly and laboratory film, followed by incubation at 30°C for 48 h. Growth was then recorded for each spot as confluent, visible, or absent. Controls included the *E. coli* strain pairs AG100-AG102 and WT-MIII (16, 49). Organic solvent tolerance was scored as no growth inhibition in the presence of 100% hexane (+), 3:1 hexane-cyclohexane (++) , 1:1 hexane-cyclohexane (+++), 1:3 hexane-cyclohexane (++++), or 100% cyclohexane (+++++). Growth inhibition was defined as a reduction in the initial inoculum of the bacterial concentration by at least two 10-fold serial dilutions in the presence of each respective organic solvent.

Statistical analysis. A Fisher exact test (two-tailed) was used to compare proportions. The Mann-Whitney U test (also two-tailed) was used to compare continuous variables. To simplify the virulence gene and antimicrobial susceptibility data for comparisons of human versus companion animal isolates, principle coordinates analysis (PCoA) was used. PCoA is a multidimensional scaling method that captures within the first two PCoA axes most of the total variance contained in a multivariable, multispecimen data set.

TABLE 2. PFGE analysis of 20 human and 9 companion animal *E. coli* ST131 isolates compared to a large reference library^a

Pulsotype(s) ^b	Present study (<i>n</i> = 29 ST131 isolates)			Reference library (<i>n</i> = 533 ST131 isolates) ^c					
	No. (%) of isolates	No. of human isolates (<i>n</i> = 20)	No. of animal isolates (<i>n</i> = 9)	Rank order in library	No. (%) of isolates	No. of human isolates	Distribution		No. of animal isolates
							Country	No.	
968	12 (41)	6	5 dogs, 1 cat	1	121 (23)	110	US	94	11 ^d
							Canada	14	
							India	1	
							Portugal	1	
800	4 (14)	4	0	2	77 (14)	77	US	55	0
							Canada	20	
							India	2	
812	3 (10)	3	0	3	29 (5)	28	US	24	1 ^d
							Canada	1	
							Spain	2	
							Portugal	1	
905	1 (3)	0	1 dog	4	18 (3)	18	US	14	0
							Canada	4	
807	1 (3)	1	0	6	6 (1)	6	US	4	0
							Canada	2	
838	1 (3)	1	0	15	3 (0.6)	3	US	3	0
920	1 (3)	0	1 dog	25	1 (0.2)	1	US	1	0
915	1 (3)	1	0	38	1 (0.2)	1	France	1	0
975	1 (3)	1	0	39	1 (0.2)	1	US	1	0
796, 976, 1110, and 1153	1 (3) each	3	1 dog ^e	NA*	NA*	NA*	NA*	NA*	NA

^a The reference library currently comprises 533 ST131 isolates from diverse locales, hosts, and clinical contexts (J. R. Johnson, unpublished data).

^b Pulsotypes are defined at the 94% Dice coefficient similarity level and are labeled sequentially as they are encountered.

^c US, United States. NA*, not applicable (these pulsotypes are represented in the reference library only by isolates from the present study); NA, not applicable (no animal isolates in pulsotype).

^d The listed reference collection animal isolates were from the United States.

^e The dog isolate was pulsotype 1153.

RESULTS

O types, confirmatory MLST, and screening for *bla*_{CTX-M-15}

The O25b *rfb* variant was detected in 211 (98.6%) of the 214 presumptive ST131 isolates, including 202 (98.5%) of the 205 human and all 9 (100%) companion animal isolates. Of the three non-O25b isolates, two were O16, and one was O157 by O-type PCR. However, seven-locus MLST confirmed the ST131 status of these three non-O25b isolates and all six (three human, three companion animal) randomly selected O25b-positive isolates. Of the 214 total isolates, only 12% (25 human, 1 canine) exhibited *bla*_{CTX-M-15}.

PFGE analysis. To assess within-clonal group and cross-species genomic commonality at a more highly resolved level, the 29 selected ST131 isolates underwent XbaI PFGE analysis (Table 2 and see also the supplemental material). Although none of the selected isolates were indistinguishable from one another, 19 (66%) exhibited >94% PFGE profile similarity to another isolate in the group and so could be considered clonal (i.e., from a multiple-isolate pulsotype). Three such multiple-isolate pulsotypes were identified (Table 2). First, 12 (6 human, 5 canine, and 1 feline) isolates (41% of 29) exhibited profiles corresponding with reference pulsotype 968, the most common pulsotype (prevalence, 23%) in an unpublished PFGE profile reference library that comprises 533 *E. coli* ST131 isolates, mainly from the United States (23) (Table 2). An additional

four human isolates matched pulsotype 800 (prevalence in reference library, 14%), and three other human isolates matched pulsotype 812 (prevalence in reference library, 5%, including one animal isolate). Six additional isolates (four human, two canine) exhibited profiles that, although representing unique pulsotypes within the present study, matched other reference library pulsotypes (prevalence in library, <3% each). Notably, for these two canine isolates, the same-pulsotype isolates within the reference library included human isolates (Table 2). The remaining four isolates (three human and one canine) each represented a novel pulsotype, i.e., were <94% similar to any study isolate or reference library isolate (Table 2). Thus, eight of nine companion animal isolates represented human-isolate-matching pulsotypes (Table 2), whereas the sole remaining canine isolate represented a novel, single-isolate pulsotype.

Virulence genotypes. Of the 53 virulence genes sought, 24 (45%) were detected among the 29 selected ST131 isolates. Aggregate virulence scores ranged from 7 to 19 (median, 12); the virulence genotype similarity ranged from 68 to 100%. Six different virulence genes were present in >90% of the 29 isolates: *fimH* (type I fimbriae, 100%), *fyuA* (yersiniabactin receptor, 100%), *usp* (uropathogenic-specific protein, 100%), *ompT* (outer membrane protease, 97%) *malX* (pathogenicity island marker, 93%), and *iutA* (aerobactin receptor, 93%). In addition, present in >75% of isolates were *sat* (secreted auto-

transporter toxin, 86%), the F10 *papA* allele (P fimbria structural subunit variant, 79%), *iha* (adhesin-siderophore receptor, 79%), and *traT* (serum resistance associated, 76%). Furthermore, present in >50% of isolates were *kpsMIII* (group 2 capsule, 66%) and *kfiC* (K5 group 2 capsule variant, 52%). Human and companion animal isolates did not differ appreciably for the prevalence of any individual virulence gene or, according to a PCoA, for aggregate virulence profile (see the supplemental material).

Susceptibility testing. Disk diffusion susceptibility testing to 19 antimicrobials showed that the 29 selected ST131 isolates were resistant to a median of 8 (range, 3 to 13) agents each (see the supplemental material). All were resistant to ampicillin, and over half, regardless of the host species, were resistant to piperacillin (90%), cephalothin (79%), streptomycin (72%), trimethoprim-sulfamethoxazole (66%), and tetracycline (59%). Human and companion animal isolates did not differ significantly for resistance prevalence for any of the antimicrobials tested. Fluoroquinolone MICs ranged from 0.5 to 128 $\mu\text{g/ml}$ for enrofloxacin (median, 64 $\mu\text{g/ml}$) and ciprofloxacin (median, 32 $\mu\text{g/ml}$), 1 to 32 $\mu\text{g/ml}$ for moxifloxacin (median, 16 $\mu\text{g/ml}$), and 0.25 to 8 $\mu\text{g/ml}$ for pradofloxacin (median, 4 $\mu\text{g/ml}$) (see the supplemental material). Human and companion animal isolates did not differ significantly for aggregate resistance profiles according to PCoA or fluoroquinolone MICs (see the supplemental material).

Detection of fluoroquinolone resistance-associated mutations in chromosomal genes. All but 1 of the 29 selected ST131 isolates had the same combination of four SNPs in the QRDRs of *gyrA* (i.e., TCG to TTG [S83L] and GAC to AAC [D87N]) and *parC* (i.e., AGT to ATT [S80I] and an atypical GAA to GTA [E84V]) (see the supplemental material). The sole exception (QUC012) had been classified as FQ^r based on its intermediate enrofloxacin susceptibility by disk diffusion, and yet it (alone among the 29 isolates) displayed full fluoroquinolone susceptibility by broth microdilution (ciprofloxacin MIC, 0.5 $\mu\text{g/ml}$) and, of the above mutations, exhibited only *gyrA* S83L. Notably, the 29 ST131 isolates also all exhibited a silent *parC* SNP, i.e., CAG instead of CAA (codon 91).

Detection of plasmid-mediated resistance genes. Among the 29 selected ST131 isolates, only 2 of 20 human isolates, versus 8 of 9 companion animal isolates, contained *qnrB* ($P < 0.001$). No isolate contained *qnrA*, *qnrS*, or *qepA*. Three human isolates but no companion animal isolates contained *aac(6')-Ib-cr* ($P > 0.05$); one of these human isolates also contained *qnrB*. The presence of *qnrB* and/or *aac(6')-Ib-cr* showed no correlation with fluoroquinolone MIC values (see the supplemental material).

Efflux pump activity. For all 29 selected ST131 isolates, the MICs of enrofloxacin, moxifloxacin, and pradofloxacin exhibited ≥ 4 -fold reductions in the presence of efflux pump inhibitor PA β N, suggesting active efflux pumps. However, only two isolates (one human, one companion animal) exhibited organic solvent tolerance, and this was only low level (i.e., "+") (see the supplemental material).

DISCUSSION

This study sought to characterize human and companion animal FQ^r *E. coli* ST131 isolates obtained from cases of extraintestinal infection in Australia and to identify the degree of

commonality across host species strains. We found that most companion animal ST131 strains showed a high degree of relatedness to Australian (and North American) human ST131 strains on the basis of PFGE profile, virulence gene content, fluoroquinolone resistance-associated chromosomal mutations, and antibiogram analyses, evidence suggesting interspecies transmission, in whichever direction. Australian ST131 isolates are of particular interest since fluoroquinolones are an important antimicrobial class that has been used in humans and pets in Australia for over 20 years (1) but was never authorized for use in Australian livestock (10).

E. coli ST131 isolates from around the world have been reported to closely resemble one another genotypically, suggesting rapid, global dissemination. This has been attributed to multiple factors, including direct human-to-human transmission (12), international travel (37), environmental dissemination (43), fecal carriage in livestock (and spread via raw meats) (46), and carriage by domestic (13, 24) or wild (15) animals. Our findings support the concept that companion animals probably participate in the dissemination of ST131 strains of human relevance.

Interestingly, our findings show that most of the studied companion animal and human ST131 isolates belong to pulsotypes present within an international ST131 strain collection, including a predominantly CTX-M-15-negative pulsotype of high prevalence in North America (pulsotype 968). Pulsotype 968 strains have been isolated previously from a dog (urine) and cohabiting cats (feces) in the United States (24). Notably, all but one of the present companion animal isolates represented a pulsotype that includes human isolates, in this collection and/or the reference library. This cross-species commonality suggests that either (i) ST131 strains are able to cross the human-companion animal species barrier or (ii) humans and companion animals can acquire ST131 strains from a common external source.

The common-source hypothesis is supported by the recent identification of FQ^r ST131 in poultry meat (32, 46), poultry feces, and extraintestinal infections in chickens (32). These poultry isolates were similar by PFGE profile and virulence gene content to human ST131 isolates from the same locality (32, 46). However, this cannot be extrapolated to the present study, since Australia has a permanent ban on the importation of fresh poultry products, and FQ^r *E. coli* have not been detected in Australian poultry (1).

Only 26 (only 12%) of our 214 study isolates (25 human, 1 canine) tested positive for *bla*_{CTX-M-15}, and the 4 CTX-M-15-positive isolates among the 29 isolates that underwent PFGE analysis represented three distinct pulsotypes. Horizontal acquisition of a CTX-M-15-encoding plasmid appears to have occurred relatively recently within ST131, subsequent to the development of fluoroquinolone resistance in ancestral ST131 clones (18). The low prevalence of *bla*_{CTX-M-15} among our study isolates may reflect either the high prevalence of the typically *bla*_{CTX-M-15}-negative pulsotype 968 (18) or a generally low prevalence of CTX-M-15 among Australian FQ^r ST131 isolates regardless of clonal background (44).

Strains of *E. coli* from phylogenetic group B2 have been shown to possess enhanced molecularly inferred virulence capacity compared to those from other *E. coli* phylogenetic groups but are generally more antimicrobial susceptible (21).

Although lacking certain traditional B2-associated virulence traits, ST131 strains still possess numerous virulence genes (23, 34) in addition to multidrug resistance. Our isolates displayed similarly extensive virulence gene profiles regardless of host species, suggesting substantial virulence potential for humans and companion animals alike irrespective of immediate source.

Since ST131 isolates have a known association with fluoroquinolone resistance, we also compared resistance mechanisms between human and companion animal isolates. Animal and human isolates possessed identical chromosomal resistance mutations and similar efflux phenotypes. The identical (and ST131-specific) pattern of *gyrA* and *parC* mutations supports the concept of clonal spread. Sequence analysis of the QRDR of *parC* revealed two distinctive nonsynonymous SNPs and one synonymous SNP, indicative of a specific *parC* genotype in the ST131 lineage. One of the nonsynonymous *parC* mutations (GAA to GTA) affecting codon 84 (E84V) is unusual for *E. coli* but was previously reported in 14 FQ^r isolates in the United States (18), evidence of its disseminated and lineage-specific nature.

In contrast to the high degree of commonality between human and companion animal ST131 isolates from Australia according to other assessed traits, the companion animal isolates exhibited a significantly higher prevalence of the plasmid-mediated *qnrB* gene. Since *qnr* genes have been previously demonstrated in multidrug-resistant *E. coli* isolates from companion animals in Australia belonging to phylogenetic groups A and D, companion animal FQ^r *E. coli* isolates may have a generally higher prevalence of these genes compared to human isolates (14). Given the (mobile) plasmidic nature of *qnrB*, this minor point of host-specific differentiation may reflect recent acquisition of *qnrB* after these strains entered the canine population. Interestingly, *qnrB* was not associated with increased fluoroquinolone MICs, evidence that among these isolates fluoroquinolone resistance is due primarily to chromosomal mutations.

Addition of the efflux pump inhibitor PAβN yielded a ≥ 4 -fold MIC reduction for enrofloxacin, moxifloxacin, and pradofloxacin in all ST131 isolates, perhaps reflecting intrinsic activity of MDR efflux pumps. However, since only two isolates showed even low-level tolerance to organic solvents (and to hexane only), the overall importance of efflux pumps as a resistance mechanism in ST131 cannot be demonstrated. Notably, of the four fluoroquinolones tested, pradofloxacin, an expanded-spectrum fluoroquinolone developed solely for the companion animal market, showed the lowest fluoroquinolone MICs (2 to 8 $\mu\text{g/ml}$).

In conclusion, we found that fluoroquinolone-resistant *E. coli* ST131 isolates from humans and companion animals in eastern Australia possess extensive similarities according to genomic backbone, virulence gene content, resistance profile, and chromosomal fluoroquinolone resistance mechanisms. Pets likely play a small but potentially significant role in ST131 transmission dynamics. ST131's high molecularly inferred virulence potential, its broad host range, and its multidrug resistance phenotype make it important for both humans and companion animals. Our findings suggest a need for ongoing surveillance of both human and veterinary antimicrobial-resistant pathogens in order to detect both the spread of existing clonal groups and the emergence of novel lineages.

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