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Phylogenetic Grouping, Antibiotic Resistance Profile, Fluoroquinolone Susceptibility and ST131 Status of Canine Extra intestinal Escherichia Coli Isolated from Submissions to a Veterinary Diagnostic Laboratory 2005-08

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Abstract

Fluoroquinolones (FQs) are a recommended treatment for Escherichia coli infections in companion animals, particularly in cases of resistance to other drug classes. In a retrospective study, 162 canine clinical E. coli isolates, obtained from veterinary diagnostic submissions (January 2005 - June 2008), were analyzed for phylogenetic group and antibiogram phenotype, using nine antimicrobials and enrofloxacin, ciprofloxacin, moxifloxacin and pradofloxacin minimum inhibitory concentrations (MICs), either in the absence or presence of an efflux pump inhibitor. The isolate susceptibility distribution was bimodal; a high proportion (141/162;87%) showed a sensitivity equivalent to wild-type E. coli (enrofloxacin MIC 0.004 - 0.06 µg/mL), while a minority (4/162;2%) showed reduced susceptibility (enrofloxacin MICs of 0.125 - 0.5 µg/mL), and the remainder (17/162;10%) yielding enrofloxacin MICs in the high-level resistance range of ≥16 µg/mL. All FQ-resistant isolates were also multidrug-resistant. The majority of FQ-resistant isolates belonged to phylogenetic group B2 (101/162;62%), and the majority of resistant isolates to group D (8/17;47%). A single resistant B2 isolate and three FQ-sensitive isolates were identified as ST131. Efflux pump activity contributed significantly to MICs for all FQs, except for ciprofloxacin, which may be attributable to its higher polarity compared to the other FQs. These findings confirm a low prevalence of FQ resistance in Australian canine E. coli isolates. Detection of a high moxifloxacin: low ciprofloxacin MIC efflux-associated phenotype (102/162;63%) amongst canine strains may indicate previous exposure to moxifloxacin selective pressure, providing more evidence of exchange of E. coli strains between humans and dogs. The presence of sensitive ST131 strains in the isolate collection does suggest, however, that resistant ST131 strains could potentially emerge under both human and veterinary antimicrobial selection pressure, a risk that could be mitigated by using the most active fluoroquinolone (i.e. pradofloxacin in dogs) against wild-type E. coli at mutant prevention concentrations.

Keywords: Extraintestinal pathogenic Escherichia coli; Antimicrobial susceptibility; Fluoroquinolone resistance; Phylogenetic group; ST131; Dogs

Introduction

Extraintestinal pathogenic Escherichia coli (ExPEC) are the main cause of urinary tract infection (UTI) in dogs, but also play a role in other extraintestinal infections [1]. In conjunction with resistance to multiple classes of antimicrobial agent, these pathogens pose a significant treatment challenge to veterinarians. A trend towards canine ExPEC strains developing FQ resistance and expressing a multidrug-resistant (MDR) phenotype [2,3] has been compounded by the recent isolation of E. coli O25b:H4 ST131 from extraintestinal infections in dogs [4] and confirmation that ST131 strains from humans and dogs appear to be closely related [5]. ST131 has shown recent and rapid global dissemination mainly in humans [5-8], but there are now several reports of its occurrence in dogs [9,10].

The development of FQ resistance is a step-wise process involving mutations in the quinolone resistance-determining region (QRDR) of the genes that encode DNA gyrase (gyrA/gyrB) and topoisomerase IV (parC/parE) [11]. In addition, chromosomally encoded MDR efflux pumps [12] and plasmid-mediated resistance genes (qnrA, qnrB, qnrC and qnrS as well as aac(6’)-Ib-cr) have been shown to contribute to reduced susceptibility [13]. Canine MDR ExPEC isolates exhibiting FQ resistance have been associated with nosocomial infections in Australian veterinary referral hospitals [14]. These isolates belonged to phylogenetic groups D and A, and possessed typical QRDR mutations, efflux pump activity and plasmid-mediated quinolone resistance genes [15,16]. Whilst the majority of more recently isolated FQ-resistant (FQ) canine clinical ExPEC from Australia (2007-2009) also were identified as phylogenetic group D [17], 10% of isolates corresponded to phylogenetic group B2, with the majority identified as virulent MDR ExPEC clonal group ST131 [5].

The isolation of FQ ST131 strains from extraintestinal infections in dogs could be explained by either of two hypotheses operating alone or in tandem. These may have originally been sensitive strains of canine origin that acquired FQ resistance under veterinary antimicrobial selection pressure, or FQ-resistant ST131 strains may have been...
directly transmitted from humans to dogs, as suggested by evidence from both basic prevalence [4] and molecular epidemiology studies [5]. In order to test these alternative hypotheses, this study retrospectively examined a collection of isolates, representing all *E. coli* obtained from cases of extraintestinal infection in dogs, submitted to an Australian veterinary diagnostic microbiology laboratory around the time of the first reports of FQ ST131 in humans (2005–2008). The isolates were anti-biogram phenotyped and susceptibility to four FQs determined by broth dilution MIC testing in the absence or presence of an efflux pump inhibitor. Isolates were phylogenetically grouped and those belonging to group B2 subjected to determination of ST131 status.

### Materials and Methods

#### Bacterial isolates

All isolates from canine extraintestinal infections in which *E. coli* was considered the primary pathogen (n = 221) were collected from Veterinary Science Diagnostic Services, The University of Queensland from January 2005 to June 2008 and stored in Brain Heart Infusion broth with 20% glycerol at -80°C until tested. Seven isolates were non-viable upon resuscitation, resulting in a total of 214 isolates. Isolates were obtained from samples from 147 dogs sourced from eight veterinary clinics in south-east Queensland. Following phylogenetic grouping and MIC testing, isolates from the same dog, with the same clinical presentation, identical phylogenetic group and antimicrobial susceptibility profile were considered repeat isolates and excluded from further analysis, leaving a total of 162 isolates for characterization.

#### Phylogenetic grouping and determination of ST131 status

All isolates underwent PCR-based determination of major phylogenetic groups (A, B1, B2 or D) by triplex PCR [18]. The phylogenetic group B2 isolates were screened for ST131 status by PCR-based detection of ST131-specific single-nucleotide polymorphisms (SNPs) in the *E. coli* housekeeping genes *ndh* (malate dehydrogenase) and *gyrB* (DNA gyrase subunit B) [19] and PCR detection of the region of the *rfb* locus (gene cluster responsible for O-type determination) specific for the O25b variant [20].

#### Antimicrobial susceptibility

All isolates underwent disc-diffusion susceptibility testing to nine antimicrobial agents: amoxicillin–clavulanic acid (AMC), ampicillin (AMP), ceftazidime (CAZ), cephalothin (KF), enrofloxacin (ENR), gentamicin (CN), tetracycline (TET), ticarcillin–clavulanic acid (TIM) and trimethoprim sulfamethoxazole (SXT), which represented classes of drug that are commonly used in companion animal veterinary practice. Resistance score was defined as the number of antimicrobials to which the isolate showed resistance, with 0.1 added to the resistance score for every antimicrobial that showed intermediate susceptibility. A resistance score of ≥ 4 was used to identify the strain as MDR.

#### Efflux pump inhibitor testing

To evaluate the contribution of efflux pumps to MIC, isolate susceptibilities to four FQs (ENR, CIP, MXF and PRA) were tested in the presence of 64 µg/mL of L-phenylalanyl-arginyl-β-naphthylamide (PAβN; Sigma P 4157) using broth microdilution [21,22], as described above.

#### Chromosomal Mutations in the Quinolone Resistance Determining Region (QRDR)

PCR amplification and Illumina sequencing (Applied Biosystems Hitachi 3130xl Genetic Analyzer) of the QRDR for the *gyrA* and *parC* genes was performed on all isolates classified as 1<sup>st</sup> or 2<sup>nd</sup>-step mutants, as previously described [25,26]. Mutations in the QRDR of each gene were determined by comparison to *E. coli* ATCC 8739.

#### Statistical methods

Fisher’s exact test was used to determine any significant associations between FQ resistance (as indicated by ENR resistance) and phylogenetic group as well as multidrug resistance and phylogenetic group.

### Results

#### Isolate epidemiology

The 162 isolates were primarily sourced from extraintestinal infections occurring in separate dogs (137/162;85%), with a small proportion of dogs (10/147;7%) yielding multiple isolates (25/162;15%). The isolates were obtained from dogs that presented to eight different veterinary hospitals in south-east Queensland, however the majority of isolates (148/162;91%) originated from two of these, which were both referral hospitals (Clinic 1,75/162;46% and Clinic 2, 73/162;45%). The majority of isolates (151/162;93%) were obtained from the urogenital tract including urine (n = 140), prostatic fluid (n = 6), the bladder wall (n = 2) and uterus (n = 3). The remaining isolates were sourced from wound infection (n = 3), liver biopsy (n = 3), peritoneal fluid (n = 1) and blood culture (n = 1). Three isolates were from an undefined aspirate. *E. coli* was isolated in pure culture on sheep blood agar and MacConkey agar plates for 70% (113/162) of samples and was considered the primary (i.e. most numerically dominant) pathogen for the remainder.

#### Phylogenetic grouping

Phylogenetic group B2 was the most represented group (102/162;63%), with approximately equal distribution of group B1 (25/162;15%) and group D (23/162;14%) and a low prevalence of group A (12/162;7%) amongst the isolates (Table 1).

#### Disc-diffusion susceptibility and MDR status

Resistance prevalence for each antimicrobial is shown in Figure 1. Although no antimicrobial agent tested showed complete efficacy against all isolates in the study, for ENR (17 isolates resistant; 10%), CAZ (15;9%) and CN (5;3%), the prevalence of resistance was ≤10%. The prevalence of resistance to SXT (23;14%), TET (27;17%), AMC (32;20%), TIM (36;22%), and KF (36;22%) was higher, with the highest proportion of isolates showing resistance to AMP (72; 44%) (Figure 1). When isolates showing intermediate susceptibility were combined with the resistant isolate fraction, the prevalence of non-susceptibility was greatly increased for AMP (72 resistant + 40 intermediate; 69%), KF (36;68;64%), TET (27;31;36%), AMC (32;18;31%) and TIM (36;6;26%), but remained low for CN (0;5;3%), CAZ (15;1;10%).
ENR (17+2;12%) and SXT (23+2;15%). The mean resistance score was 1.7, with a median score of 1 (range 0 to 8). Twenty-five isolates (15%) showed complete susceptibility to all tested antimicrobials, and 32 isolates (20%) were classified as MDR. All ENR resistant isolates except one qualified as MDR (mean resistance score 6.24; range 1.3 to 8).

Associations of phylogenetic group with FQ resistance and MDR status

Phylogenetic groups A and D contained the majority of isolates exhibiting ENR resistance (group A: 5/17; 29%; group D: 8/17; 47%) and only a single group B2 isolate (1/101; 1%) exhibited this phenotype (Table 1). Only 11% (16/145) of isolates shown to be ENR-sensitive qualified as MDR, whereas ENR resistant isolates were much more likely to also be MDR (16/17; 94%, P < 0.001). Of the MDR isolates, the majority belonged to phylogenetic group D (13/32; 41%), whilst the prevalence of MDR in the other phylogenetic groups was much lower (group B1: 8/32; 25%; group B2: 6/32; 19%; group A: 5/32; 16%).

Resistant phenotypes amongst group B2 and D isolates classified as MDR were varied, though AMC:AMP:C2:KF:TIM (5/19) and AMC:AMP:KF:ENR:TET:TIM:SXT (3/19) were shared by more than a single isolate. The single ENR-resistant group B2 strain had the following profile: AMP:KF:ENR:CN. Significant differences in isolate proportional distribution across phylogenetic group were evident between ENR-resistant/MDR and sensitive isolates (Table 1), with a trend towards increased representation for groups A and D and decreased representation for group B2 for resistant isolates.

ST131 status

The single ENR-resistant phylogenetic group B2 isolate and 3/101 (3%) ENR-sensitive group B2 isolates were shown to belong to clonal group ST131. Two of these were positive for the O25b rfb, rfb i.e., one ENR-resistant, one ENR-sensitive. The three FQ-sensitive ST131 isolates exhibited ENR resistance (group A: 5/17; 29%, group D: 8/17; 47%).

MIC determination

Prevalence of resistance by MIC was the same across the four tested FQs, with the same 17 isolates classified as FQ by disc diffusion also exhibiting resistance on the basis of MIC testing. The remaining isolates were fully sensitive (145/162;90%) and no intermediate isolates were detected (Figure 2). Most isolates were considered to be wild-type (141/162;87%) with just four isolates displaying MICs equivalent to 1<sup>st</sup> or 2<sup>nd</sup>-step FQ-resistant mutants (three isolates with enrofloxacin MICs of 0.125 µg/mL and one isolate with a MIC of 0.5 µg/mL). All remaining isolates (n = 17) displayed high-level resistance. For the two isolates classified as showing intermediate susceptibility to ENR by disc diffusion, both were sensitive (ENR MICs of 0.06 µg/mL and 0.5 µg/mL) on the basis of MIC testing against all four FQs. MIC values were the lowest for PRA (MIC median 0.015 µg/mL, range 0.004 to 16 µg/mL vs. ENR MIC median 0.03 µg/mL, range 0.004 to 512 µg/mL). The MIC values for CIP vs. MFX showed a four-fold or greater difference in 63% (102/162) of the isolates. The range, MIC<sub>50</sub> and MIC<sub>90</sub> values for the 162 isolates are shown in Table 2.

Efflux pump inhibitor testing

Phenotypic expression of an efflux pump was observed in the majority of <i>E. coli</i> isolates (Figure 3). In the presence of an efflux pump inhibitor, a 2-4-fold decrease in MIC was seen in 157 (97%) of isolates against ENR (range 2 to 64-fold; median 8-fold), 146 (90%) of isolates against PRA (range no change to 32-fold; median 8-fold) and 153 (94%) of isolates against MXF (range no change to 64-fold; median 8-fold) (Table 2). However, only 30 (19%) isolates showed a decrease in CIP MIC in the presence of efflux pump inhibitor (range no change to 8-fold; median 2-fold). These efflux phenotypes were consistent for both sensitive and resistant isolates, discriminating between CIP and the other FQs.

Chromosomal mutations in the QRDR

In two of the four isolates classified as 1<sup>st</sup> or 2<sup>nd</sup>-step mutants (ENR MICs, 0.125 µg/mL and 0.5 µg/mL), identical FQ resistance-associated mutations were detected in the QRDR of gyrA, i.e., gyrA 83L (TCG to TTG) compared to <i>E. coli</i> ATCC 8739. The remaining two isolates displayed no mutation in the QRDR of gyrA (ENR MICs, 0.125 µg/mL for both) and none of the four isolates displayed mutations in the

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### Table 1: Phenotypic grouping of 162 canine clinical <i>E. coli</i> isolates by enrofloxacin sensitivity (top) and MDR status (bottom).

<table>
<thead>
<tr>
<th>Phylogenetic Group</th>
<th>Number of enrofloxacin sensitive isolates (%)</th>
<th>Number of enrofloxacin resistant isolates (%)</th>
<th>P value (sensitive vs resistant)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 (5)</td>
<td>5 (29)</td>
<td>0.004</td>
<td>12 (7)</td>
</tr>
<tr>
<td>B1</td>
<td>22 (18)</td>
<td>3 (18)</td>
<td>0.792</td>
<td>25 (15)</td>
</tr>
<tr>
<td>B2</td>
<td>101 (70)</td>
<td>1 (6)</td>
<td>&lt; 0.001</td>
<td>102 (63)</td>
</tr>
<tr>
<td>D</td>
<td>15 (10)</td>
<td>8 (47)</td>
<td>&lt; 0.001</td>
<td>23 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>145 (100)</td>
<td>17 (100)</td>
<td></td>
<td>162 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phylogenetic Group</th>
<th>Number of non-MDR isolates (%)</th>
<th>Number of MDR isolates (%)</th>
<th>P value (MDR vs non-MDR)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 (5)</td>
<td>5 (16)</td>
<td>0.059</td>
<td>12 (7)</td>
</tr>
<tr>
<td>B1</td>
<td>17 (13)</td>
<td>8 (25)</td>
<td>0.102</td>
<td>25 (15)</td>
</tr>
<tr>
<td>B2</td>
<td>96 (73)</td>
<td>6 (19)</td>
<td>&lt; 0.001</td>
<td>102 (63)</td>
</tr>
<tr>
<td>D</td>
<td>10 (8)</td>
<td>13 (41)</td>
<td>&lt; 0.001</td>
<td>23 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (100)</td>
<td>32 (100)</td>
<td></td>
<td>162 (100)</td>
</tr>
</tbody>
</table>

### Table 2: Range, MIC<sub>50</sub> and MIC<sub>90</sub> for four fluoroquinolones in the presence and absence of efflux pump inhibitor (EPI).

<table>
<thead>
<tr>
<th>Antimicrobial +/- EPI</th>
<th>Enrofloxacin</th>
<th>Ciprofloxacin</th>
<th>Moxifloxacin</th>
<th>Pradofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-EPI</td>
<td>0.004-512</td>
<td>&lt;0.001-32</td>
<td>0.002-256</td>
<td>0.002-128</td>
</tr>
<tr>
<td>+EPI</td>
<td>0.008-128</td>
<td>&lt;0.001-32</td>
<td>0.002-128</td>
<td>0.002-168</td>
</tr>
</tbody>
</table>

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isolates from extraintestinal infections in Australian dogs belong to phylogenetic group B2 and the majority of FQ isolates to group D. Prior to the global emergence of ST131, similar phylogenetic group distributions were observed in both sensitive and resistant ExPEC isolates of human origin [27-29].

Clonal group ST131, belonging to phylogenetic group B2, became recognized initially due to the presence of an extended spectrum β-lactamase gene (bla<sub>CTX-M-15</sub>) and an association with multidrug resistance [6-8]. Later, a stronger association to FQ resistance was found, particularly outside of Europe [19], indicating FQ resistance may have aided the successful dissemination of this clonal group. In the present study, only three sensitive canine B2 isolates were identified as ST131, together with a single resistant B2 isolate, all isolated in the latter part of the study (between 2007 and 2008). These results suggest that both sensitive and resistant ST131 strains are circulating within the canine population. Only one of the three FQ-sensitive ST131 isolates was classified as MDR and all shared a wild-type FQ susceptibility phenotype as well as showing susceptibility to SXT. The close genetic similarity of Australian human and canine FQ ST131 isolates, with a high proportion sharing clonal commonality with a prominent international ST131 pulstype [5], indicates the possibility of frequent human-dog exchange of ST131 isolates. Whilst humans can be considered to be the primary reservoir of FQ ST131 strains [30], the finding of FQ-sensitive ST131 strains in dogs, albeit at low prevalence, may indicate a role for veterinary antimicrobial selection pressure in the emergence of FQ ST131 variants. Studies to determine the prevalence of FQ sensitive ST131 strains in the human population are also warranted as alternatively, ST131 strains, regardless of their sensitivity profile, could be readily transferred between humans and dogs. In addition, the sensitive canine ST131 strains identified in the present study should be compared with their FQ counterparts as well as FQ-sensitive and FQ human ST131 strains to determine phylogenetic and pathotypic similarities.

The prevalence of ST131 in E. coli isolate collections from humans is only now being fully investigated. Previously, studies have mainly focused on collections of ESBL-positive and/or FQ isolates due to their positive associations with ST131, and initial data suggested that FQ-sensitive strains were extremely rare [19]. However, two recent studies have provided evidence that FQ-sensitive ST131 isolates may account for significant proportions of isolates. Blanco et al. [32] examined 500 human E. coli isolates from extraintestinal infections, predominately from urine, in Spain and found ST131 accounted for 12% (59/500). Although the majority of these (41/59,69%) were FQ-sensitive, nearly a third of the isolates (18/59,31%) showed FQ sensitivity. A similar prevalence was observed by Gibreel et al. [32] who examined 300 UTI-associated human E. coli isolates from England and found ST131 also accounted for 12% of the collection (37/300), with 35% (13/37) displaying FQ sensitivity. In our canine study the overall ST131 prevalence was 4% (15/370) with 35% (5/14) displaying FQ sensitivity. A similar prevalence was observed by Gibreel et al. [32] who examined 300 UTI-associated human E. coli isolates from England and found ST131 also accounted for 12% of the collection (37/300), with 35% (13/37) displaying FQ sensitivity. In our canine study the overall ST131 prevalence was 4% (15/370) with 35% (5/14) displaying FQ sensitivity.
is not necessarily an indicator of FQ resistance, with over 20% of the isolate collection classified as MDR, but only 10% as FQR. Therefore, although treatment may have failed for many antimicrobial classes, FQs may still be an effective treatment choice. We collected clinical ExPEC isolates over a three and a half year timespan and the most predominant site of isolation was the urogenital tract (93% of cases), confirming the importance of E. coli as the major causative agent of both simple and complicated cases of UTI in dogs [1] and associated urogenital infections [36].

A large proportion of veterinary ExPEC isolates from dogs are usually resistant to AMP, TET and KF [37] and this was supported by the high rate of resistance to these antimicrobial classes (<65% susceptibility) in our study. However, the majority of isolates (i.e., >85%) were still susceptible to FQs, SXT and CN, which is also consistent with other studies [37,38]. It is difficult to compare international data on E. coli resistance in companion animals due to the differences in study design and data presentation, particularly before the first veterinary CLSI guidelines became available in 1999 [39]. However, studies have continually shown that FQ susceptibility has remained high for companion animal isolates as shown by Cohn et al. [40] (approx. 482/547;88%), Oluoch et al. [37] (571/652;88%) and Shaheen et al. [34,38] (304/376;81%), all showing >80% susceptibility compared to 90% susceptibility in our isolate collection. Furthermore, the majority of isolates characterized in the present study can be considered to be wild-type and not likely to possess mutations in the QRDRs of gyrase and/or topoisomerase target genes.

The higher prevalence of FQ susceptibility observed in our study may relate to possible overestimations in previous studies as isolates from repeat samples from the same animal may have been included, since all E. coli isolates were analyzed without reference to patient identification, or that our study location was geographically restricted with isolates predominantly sourced from two referral hospitals. However, as referral hospitals are more likely to treat dogs with previous or current history of antimicrobial treatment and/or underlying refractory illness, the prevalence of resistance to FQs would likely be higher than in primary accession practices, resulting in lower FQ susceptibility rates. Our findings may also reflect the unique epidemiology of FQ resistance in companion animals in Australia, given its geographic isolation and the ban on the use of FQs in food-producing animals.

As regularly observed in Australian clinical studies involving both human [41] and veterinary isolates [42], our canine ExPEC collection showed a bimodal distribution of FQ susceptibility, with the majority of isolates fully sensitive, very few isolates of intermediate susceptibility and a small proportion of isolates displaying high-level resistance. Bimodal distribution is of particular interest as this appears to be an unusual characteristic particularly of this antimicrobial class. Bimodal distribution of this kind will affect both MIC₅₀ and MIC₉₀ values, which are regularly used by clinicians when making appropriate antimicrobial choices and therefore is of particular note. FQ susceptibility patterns were equivalent across all four FQs tested, with 17 isolates showing resistance and PRA showing the lowest MICs in both sensitive and resistant isolates. Given PRA’s dual targeting features, low mutant prevention concentrations [23], reduced mutant selection windows [24], favorable pharmacokinetics [43] and the fact that it would...
effectively eliminate both wild-type and first-step mutants (87% and 1.8% of isolates, respectively, characterized in the present study) at the recommended dosing, PRA should be used in preference to older veterinary FQs for the treatment of canine urinary tract infections [44]. However, once resistance to one FQ is identified, it tends to apply to most compounds within the class. Although no CLSI guidelines have been set for PRA, when a provisional breakpoint of 2 µg/mL was applied, all isolates that showed resistance to ENR, CIP and MXF also qualified as “resistant” to PRA.

Fluoroquinolone resistance is conferred through various combinations of mechanisms, including mutations in target enzymes, reduced expression of outer membrane porins, drug efflux, and plasmid-mediated mechanisms [13]. Chromosomal mutations in the target enzymes are likely to be present at MICs >0.125 µg/mL and can escalate to several (generally two) point mutations in both gyrA and parC once the isolate is considered FQ. In the present study, sequencing of the QRDRs of gyrA and parC was undertaken for isolates considered 1st and 2nd-step mutants in order to clarify the contribution of point mutations to FQ resistance. Two out of the four isolates in this category showed single mutations in the QRDR of gyrA (S83L) and no isolate had a mutation in the QRDR of parC. The low prevalence of 1st and 2nd-step mutants amongst our clinical ExPEC strains (2/162;1%) is also in agreement with other studies [41,48].

The unchanged MICs of CIP in the presence of the efflux pump inhibitor, PAβN, versus 2-4-fold reductions in MICs observed for ENR, MXF and PRA, most likely indicates differential activation of FQ efflux systems as well as a significant contribution of efflux in defining the actual phenotypes encountered. Overall differences in the chemical structures [24] and/or lipophilicity between CIP and ENR, MXF and PRA, with lipophilicity being reflected by their octanol:water coefficients (P<) determined at neutral pH, help to explain this phenomenon. The respective log P< values amounted to -1.5 for CIP as compared to 0.36 for ENR [47] and to -1.9 and -1.4 for MXF and PRA, respectively [48,49]. Differences in chemical structure and lipophilicity should cause activation of distinct separate efflux systems, thus providing for the observed phenotypes and the difference in efficacy of PAβN between CIP and the other three FQs. Another example for the primary role of efflux systems in defining the FQ phenotype, this time in the presence of ENR, MXF or PRA, but not CIP.

The unchanged MICs of CIP in the presence of the efflux pump inhibitor, PAβN, versus 2-4-fold reductions in MICs observed for ENR, MXF and PRA, most likely indicates differential activation of FQ efflux systems as well as a significant contribution of efflux in defining the actual phenotypes encountered. Overall differences in the chemical structures [24] and/or lipophilicity between CIP and ENR, MXF and PRA, with lipophilicity being reflected by their octanol:water coefficients (P<) determined at neutral pH, help to explain this phenomenon. The respective log P< values amounted to -1.5 for CIP as compared to 0.36 for ENR [47] and to -1.9 and -1.4 for MXF and PRA, respectively [48,49]. Differences in chemical structure and lipophilicity should cause activation of distinct separate efflux systems, thus providing for the observed phenotypes and the difference in efficacy of PAβN between CIP and the other three FQs. Another example for the primary role of efflux systems in defining the FQ phenotype, this time in the presence of an efflux inhibitor, was the observed low MIC values of CIP versus increased MICs (i.e. greater than four-fold) for MXF in several studies involving human clinical isolates of E. coli, which, however, were left uncommented [50,51,52] , but would indicate the presence of a moxifloxacin-specific efflux. Detection of this particular efflux phenotype in the majority (63%) of our canine isolates may indicate that these isolates had been exposed to MFX selective pressure, thus providing further evidence of potential human to dog transmission of ExPEC strains.

In conclusion, this study identified a low prevalence of ST131 in a canine clinical E. coli isolate collection from Australia, but demonstrated the presence of both FQ-sensitive and FQ-resistant variants of this B2 clonal group. Whilst humans can be considered to be the primary reservoir of FQ ST131, antimicrobial selection pressure in the veterinary field could play a role in the emergence of FQ ST131 strains in dogs. The low prevalence of both FQ resistant and 1st and 2nd step mutants showing reduced FQ susceptibility in Australian canine ExPEC isolates, and the demonstrated low MICs of pradofloxacin support its use compared to other FQs when indicated for canine urogenital infections. Differing efflux phenotypes were observed in all isolates and contributed to increased FQ MIC for all tested FQs apart from CIP, which may be related to its chemical structure and octanol:water coefficient.

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