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Characterization of antimicrobial-resistant *Escherichia coli* causing urinary tract infections in dogs: Passive surveillance in Saskatchewan, Canada 2014 to 2018

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Abstract

Background: Urinary tract infections (UTIs) are common in dogs and can be caused by multidrug-resistant *Escherichia coli* (*E coli*).

Objective: To describe the frequency and mechanisms of antimicrobial resistance (AMR) among *E coli* causing UTIs in dogs in Western Canada during a 4-year surveillance period.

Animals: Urine from 516 dogs.

Methods: From November 2014 to 2018, 516 nonduplicate *E coli* isolates from the urine of dogs were collected from a diagnostic laboratory. Susceptibility testing was determined for a panel of 14 antimicrobials belonging to 7 drug classes. Resistant isolates were screened for the presence of extended-spectrum beta-lactamases (ESBLs), AmpC β-lactamases, and plasmid-mediated quinolone resistance (PMQR) genes. Epidemiological relationships were assessed by MLST.

Results: 80.2% (414/516) of isolates were susceptible to all antimicrobials tested. There was no significant increase in the proportion of isolates resistant to any of the tested antimicrobials during the study period. Resistance to ampicillin was the most common (14.9%, 77/516). Overall, 12 isolates had bla_{CMY-2}-type AmpC β-lactamases, and 7 produced CTX-M-type ESBLs. A single isolate had the *aac(6′)-Ib-cr* PMQR gene. The *qnr* and *qep*A determinants were not detected. A single isolate belonging to the pandemic lineage ST131 was identified.

Conclusion: *Escherichia coli* isolated from the urine of dogs in our region remain susceptible to first-line therapies, though resistance, particularly to the aminopenicillins, warrants monitoring. This is the first description of *E coli* ST131 from a companion animal in Canada.

KEYWORDS
bacterial, gram-negative bacteria, microbiology, multidrug resistant, renal

Abbreviations: AMR, antimicrobial resistance; *E coli*, *Escherichia coli*; ESBL, extended-spectrum beta-lactamase; MDR, multidrug resistance; PMQR, plasmid-mediated quinolone resistance; UTI, urinary tract infection.

1 | INTRODUCTION

Antimicrobial resistance (AMR) has become a common concern of animal and human health care professionals. Multidrug resistant (MDR) infections are associated with increased mortality and cost of care.[1] In dogs, the urinary tract is the most common extraintestinal site for MDR *Escherichia coli*.[2] Furthermore, the close contact between humans and their pets has implications for the sharing of resistant commensal and pathogenic bacteria between species. Although the exact role of dogs in this transmission cycle has not been adequately studied, there is evidence of "strain sharing."[3,4]

Uncomplicated or sporadic urinary tract infections (UTIs) occur in approximately 14% of dogs that visit a veterinarian in the dog's lifetime, representing a frequent reason for the prescription of antimicrobials.[5] The Gram-negative bacterium *E coli* is the most frequent cause of UTIs, comprising approximately 50% of positive canine urine cultures.[6] Currently, the International Society for Companion Animal Infectious Diseases (ISCAID) recommends amoxicillin or trimethoprim + sulfamethoxazole as first-line empirical treatments for these infections.[7,8] Broader spectrum drugs, such as the fluoroquinolones, chloramphenicol, doxycycline, fosfomycin, and nitrofurantoin, should be reserved for cases where laboratory results indicate a lack of susceptibility to empirical treatments.[8]

Some of the most troubling emerging resistance mechanisms in *E coli* include the extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and the PMQR genes.[9,10] The ESBLs are capable of hydrolyzing extended-spectrum β-lactams including the third-generation cephalosporins; since the beginning of the 21st century, the CTX-M-type enzymes have become the most common.[9,10] Currently, the most widely distributed CTX-M alleles are $bla_{\text{CTX-M-1,–15 and –14}}$, which have also been isolated from infected and colonized companion animals.[9,10] The AmpC-type β-lactamases confer resistance to cefoxitin, in addition to extended spectrum cephalosporin resistance and are not inhibited by clavulanic acid.[10,11] $bla_{\text{CMY-2}}$ is the most common type detected in companion animals, identified in dogs from Japan, Denmark, and Canada.[9,12-14] Three types of transmissible quinolone resistance genes occur and are target protection proteins (*qnr*A, *qnr*B, *qnr*C, *qnr*D, and *qnr*S), inactivating enzyme *aac(6′)-Ib-cr*, and efflux pumps *qep*A and *oqx*AB.[9] The tendency of the *aac(6′)-Ib-cr* gene to colocate on plasmids harboring ESBLs is of particular concern, as it provides the framework for the emergence of MDR.[9]

The emergence of transmissible AMR among *E coli* isolated from the urine of dogs in other regions warrants an investigation into the prevalence and mechanisms of resistance in this pathogen in Canada. Previous work out of Saskatchewan detected a particularly low frequency of resistance (~80% pan susceptibility) among the study sample of *E coli* isolated from the urine of dogs.[12] These baseline findings provide a unique opportunity to detect the emergence of clinically relevant resistance determinates in this region. This study aims to describe the frequency and mechanisms of AMR among *E coli* causing canine UTIs in Western Canada during a 4-year surveillance period.

2 | MATERIALS AND METHODS

2.1 | Microbiological analysis and inclusion criteria

Diagnostic urine samples from dogs submitted to Prairie Diagnostic Services (PDS) in Saskatoon, SK, were collected from October 2014 to 2018. Initial identification was by biochemical testing, including indole, triple sugar iron (TSI) agar, urea, and citrate (2014-2015); and matrix-assisted laser desorption/ionization time of flight (2015-2018). In cases of suspicious colony morphology or contamination, bacterial identity was confirmed by lactose fermentation, the spot indole test or phylogenetic analysis of universal bacterial targets (*16S rRNA* or *cpn60*). In order to be included in the study, isolates had to be confirmed as first-time submissions of canine urine with a positive *E coli* culture from 1 of the 4 western Canadian provinces. A total of 194 isolates were excluded from analysis over the study period (Table 1). Geographic information regarding the origin of each submission (client province of origin) was also recorded.

2.2 | Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by broth microdilution using the Sensititre system, and the Gram-negative panels (CMV3AGNF/CMV4AGNF) were used. Agents tested include ampicillin, amoxicillin-clavulanate, cefoxitin, ceftriaxone, ceftiofur, meropenem, nalidixic acid, ciprofloxacin, gentamicin, tetracycline, chloramphenicol, sulfisoxazole, trimethoprim-sulfamethoxazole, and azithromycin. Results were interpreted according to Clinical and Laboratory Standards Institute breakpoints.[15] Multidrug resistance was defined as resistance to at least 1 antimicrobial in 3 or more classes, all β-lactams were considered to be 1 class for the purposes of this analysis.[16]

TABLE 1 An overview of excluded isolates (n = 194)

Exclusion criteria	Number of isolates
Not urine	28
Ear/skin swab	10
Feces	3
Other	15
Not canine	24
Equine	1
Feline	21
Porcine	2
Not *E coli*	15
Citrobacter spp.	3
Enterobacter spp.	1
Enterococcus spp.	6
Pseudomonas spp.	1
Staphylococcus pseudintermedius	4
Repeat submissions	127

TABLE 2 Geographic distribution of isolates (n = 516)

Province	Year 1	Year 2	Year 3	Year 4	Total
Alberta (n = 18)	3	6	2	7	3.5%
British Columbia (n = 1)	0	0	0	1	0.2%
Manitoba (n = 18)	3	1	6	8	3.5%
Saskatchewan (n = 479)	80	139	124	136	92.8%

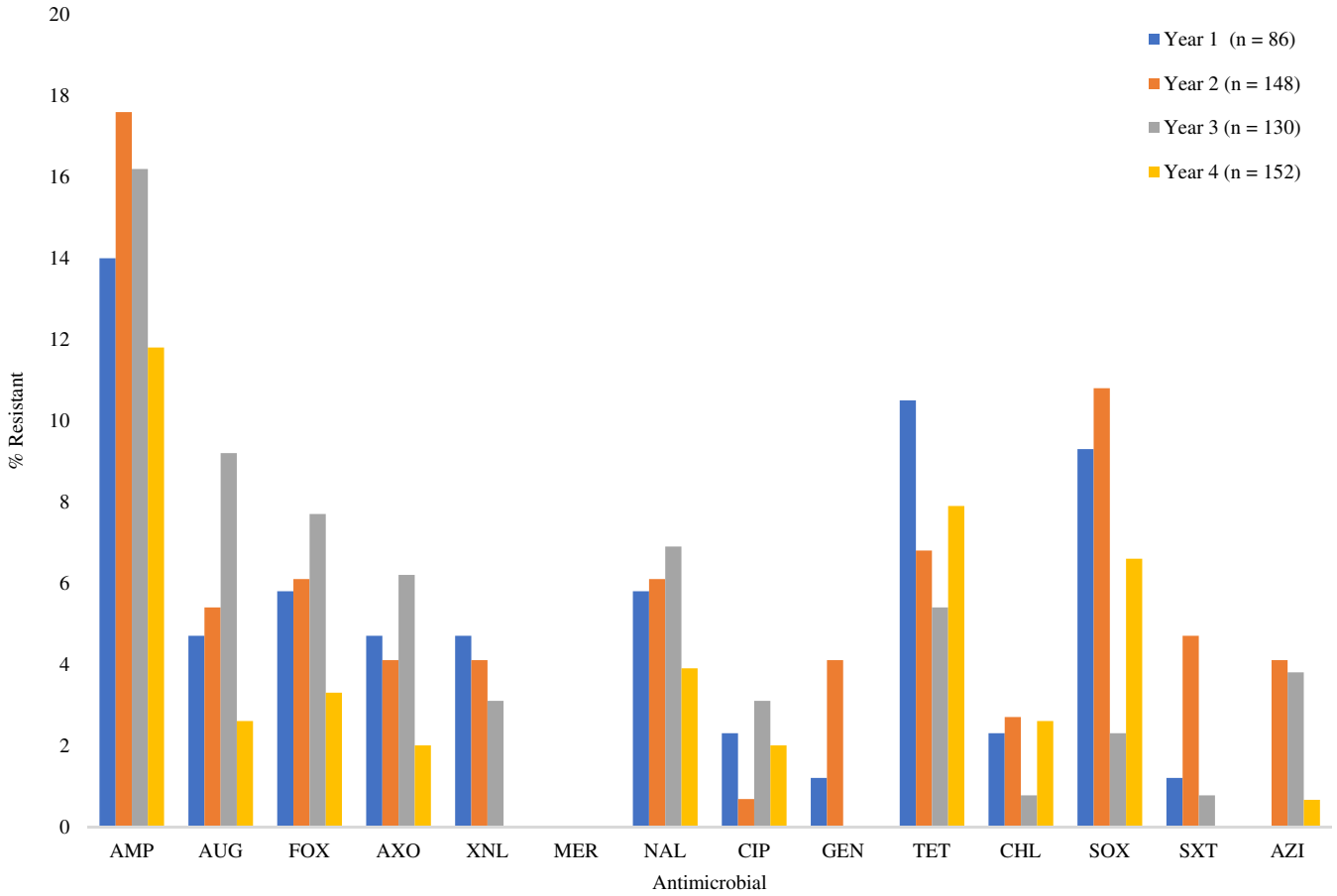

FIGURE 1 Number of isolates (n = 516) exhibiting resistance across 4 years of a canine urinary *E coli* antimicrobial resistance surveillance program. AMP, ampicillin; AUG, amoxicillin-clavulanate; AXO, ceftriaxone; AZI, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; FOX, cefoxitin; GEN, gentamicin; MER, meropenem; NAL, nalidixic acid; SOX, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; and XNL, ceftiofur

2.3 | Detection of resistance genes

All third-generation cephalosporin resistant isolates were initially screened using universal primers for CTX-M type genes. Any positive isolates were sequentially screened for CTX-M groups 1, 2, 8, and 9 as previously described.[17] Isolates were additionally screened for the SHV-and TEM-type ESBLs.[18,19] Cephamycin-resistant isolates were screened for bla_{CMY-2}-type AmpC β-lactamase genes.[17] Nalidixic acid and ciprofloxacin-resistant isolates were screened for each of the PMQR determinants; *qnr*A, *qnr*B, *qnr*S, *aac(6′)-Ib-cr*, and *qep*A.[20-22] PCR products were then sequenced by Macrogen Inc. The identity of sequences was determined by comparison with the National Center for Biotechnology Information and the Comprehensive Antibiotic Resistance Databases using a basic local alignment search tool.

2.3.1 | Typing of ESBL- and PMQR-producing isolates

To identify resistant strains and to determine whether ESBL and PMQR producers were clonal, we utilize the MLST scheme for *E coli* described by the Warwick Institute (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). For each isolate, 7 housekeeping genes (*adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A, and *rec*A) were amplified and compared to the pubMLST database.

TABLE 3 Annual frequency of resistance by drug (%R; [MIC_{50}, MIC_{90} in μg/mL])

Antimicrobial	Year 1	Year 2	Year 3	Year 4
AMP	14.0% (4, 32)	17.6% (4,64)	16.2% (4, 32)	11.8% (4, 32)
AUG	4.7% (4, 8)	5.4% (4, 8)	9.2% (4, 8)	2.6% (4, 8)
FOX	5.8% (4, 8)	6.7% (4, 8)	7.7% (4, 16)	3.3% (4, 8)
AXO	4.7% (0.25, 0.25)	4.1% (0.25,0.25)	6.1% (0.25, 0.25)	2.0% (0.25, 0.25)
XNL	4.7% (0.5, 1.0)	4.1% (0.5,1)	3.1% 0.5, 1)	0% (n/a)
MER	0% (0.06, 0.06)	0% (0.06, 0.06)	0% (0.06, 0.06)	0% (0.06, 0.06)
NAL	5.8% (2, 4)	6.1% (2, 4)	6.9% (4, 8)	3.9% (2, 4)
CIP	2.3% (0.015, 0.03)	0.7% (0.015, 0.03)	3.1% (0.03, 0.06)	2.0% (0.03, 0.03)
GEN	1.2% (1, 2)	4.1% (0.5,1)	0% (0.5,1)	0% (1)
TET	10.5% (4)	6.8% (4)	5.4% (4, 8)	7.9% (4, 8)
CHL	2.3% (8, 16)	2.7% (8, 16)	0.8% (16)	2.6% (8, 16)
SOX	9.3% (16128)	10.8% (32512)	2.3% (16, 32)	6.6% (16,64)
SXT	1.2% (0.12, 0.12)	4.7% (0.12, 0.12)	0.8% (0.12, 0.12)	0% (0.12, 0.12)
AZI	0% (4, 8)	4.1% (4, 8)	3.8% (4, 8)	0.7% (4, 8)

2.4 | Statistical analysis

The relationship between the proportion of resistant and susceptible isolates to a given antimicrobial and the year of submission was assessed using the Fisher's exact test with Bonferroni's correction ($P < .01$). The change in the proportion of MDR and pan susceptible isolates was examined using the Fisher's exact test with Bonferroni correction ($P < .01$). The relationship between β-lactamase production and the phenotypes MDR and fluoroquinolone resistance were also assessed using a Fisher's exact test ($P < .01$). Statistical analyses were performed using the GraphPad software.

3 | RESULTS

3.1 | Microbiological analysis and inclusion criteria

From November 1, 2014 to October 31, 2018, 710 *E coli* isolates were collected. A total of 194 isolates did not meet the inclusion criteria of this study and were therefore excluded. The most common reason for exclusion was a repeat isolate from the same dog (n = 127) (Table 1). Of the 516 included isolates, 92.8% (n = 479) originated from Saskatchewan, while the remaining 7% was split equally between Manitoba (n = 18) and Alberta (n = 18). A single isolate was submitted from British Columbia (n = 1; Table 2).

3.2 | Antimicrobial susceptibility testing

There was no statistically significant increase in the frequency of resistance to any of the 14 antimicrobials tested across the study period (Figure 1, Table 3). There was a statistically significant decrease in the proportion of isolates resistant to sulfisoxazole and SXT observed during the study period ($P < .01$) between years 2 and 3 ($P < .01$) and years 2 and 4 ($P < .01$), respectively. Overall, 80.2% (414/516) of isolates were susceptible to all antimicrobials tested, while 6.0% were MDR. There was no change in the frequency of pan-susceptibility or MDR over the study period. Susceptibility remained above 90% for all agents tested except ampicillin (85% 439/516 susceptible). After ampicillin, resistance to tetracycline (7.4%, 38/516), sulfisoxazole (7.2% 37/516), nalidixic acid (5.6%, 29/516), cefoxitin (5.6%, 29/516), and amoxicillin-clavulanate (5.4%, 28/516) were most common. The frequency of resistance for the remaining antimicrobials was less than 5%. Meropenem resistance was not detected. The majority of isolates resistant to a single antimicrobial class were resistant to β-lactams (73%). Of the MDR isolates, the majority of phenotypes included resistance to β-lactams (93.5%, 29/31) and/or tetracyclines (80.6%, 25/31). A single isolate was resistant to all 7 antimicrobial classes tested.

3.3 | Detection of resistance genes

Of 516 isolates, 4.3% (22/516) exhibited resistance to the third-generation cephalosporins ceftriaxone or ceftiofur. Of the ESBLs detected, $bla_{CTX\text{-}M\text{-}27}$ was the most common (n = 3) followed by $bla_{CTX\text{-}M\text{-}15}$ (n = 2) and $bla_{CTX\text{-}M\text{-}14}$ (n = 2). One of the $bla_{CTX\text{-}M\text{-}27}$ producers displayed resistance to all 7 antimicrobial classes tested (Table 4). There was no significant change in the frequency of CTX-M-producing isolates observed over the study period. SHV- and TEM-type ESBLs were not detected. Of 22 third-generation cephalosporin-resistant isolates, 68% (15/22) were also cefoxitin resistant (MIC ≥ 32), of which 12 were positive for $bla_{CMY\text{-}2}$ (Table 4). There was no significant change in the frequency of $bla_{CMY\text{-}2}$-producing isolates across the study period. The overall occurrence of AmpC β-lactamases and ESBLs in our isolates collection was 2.3% (n = 12) and 1.3% (n = 7), respectively. Broad spectrum β-lactamase production (CTX-M- or AmpC-type enzymes) was significantly associated with MDR ($P < .01$) and fluoroquinolone resistance ($P < .01$). The PMQR determinant *aac(6′)-Ib-cr* was identified in a single isolate that was MDR to 4 antimicrobial classes. However, this isolate did not possess 1 of the broad-spectrum β-lactamases included in this study.

TABLE 4 Phenotypic and genotypic characteristics of third-generation cephalosporin resistant *E coli* isolated from the urine of dogs

Isolate	Year	Phenotype	Genotype
ECPDS140	1	AMP + AUG + FOX + AXO + XNL + GEN + TET + CHL + SOX	bla_{CMY-2}
ECPDS155	1	AMP + AUG + FOX + AXO + XNL + TET + CHL + SOX	bla_{CMY-2}
ECPDS202	1	AMP + AXO + XNL + NAL + TET + SOX + SXT	$bla_{CTX-M-15}$
ECPDS218[a]	2	AMP + AUG + FOX + AXO + XNL + NAL + CIP + TET + SOX	*aac(6′)- Ib-cr*
ECPDS236	2	AMP + AXO + XNL	$bla_{CTX-M-15}$
ECPDS264	2	AMP + AUG + FOX + AXO + TET + SOX	bla_{CMY-2}
ECPDS272	2	AMP + AUG + FOX + AXO + XNL + GEN + TET + SOX	bla_{CMY-2}
ECPDS289	2	AMP + AUG + FOX + AXO + XNL + NAL + CIP + TET + SOX	bla_{CMY-2}
ECPDS306	2	AMP + AUG + FOX + XNL + NAL + GEN + SOX	bla_{CMY-2}
ECPDS318	2	AMP + AXO + XNL + NAL + GEN + TET + CHL + SOX + SXT + AZI	$bla_{CTX-M-27}$
ECPDS426	2	AMP + AUG + FOX + AXO + XNL	bla_{CMY-2}
ECPDS462	3	AMP + AUG + FOX + AXO + XNL	bla_{CMY-2}
ECPDS464[a]	3	AMP + AUG + FOX + AXO + XNL + AZI	—
ECPDS465[a]	3	AMP + AUG + FOX + AXO + XNL	—
ECPDS494	3	AMP + AXO + XNL + NAL + CIP + TET + CHL + AZI	$bla_{CTX-M-14}$
ECPDS537	3	AMP + AUG + FOX + AXO + NAL + TET	bla_{CMY-2}
ECPDS538	3	AMP + AUG + FOX + AXO + AZI	bla_{CMY-2}
ECPDS552	3	AMP + AXO + NAL + CIP + TET + SOX + SXT + AZI	$bla_{CTX-M-27}$
ECPDS580	3	AMP + AUG + FOX + AXO + NAL	bla_{CMY-2}
ECPDS682	4	AMP + AUG + FOX + AXO	bla_{CMY-2}
ECPDS698	4	AMP + AXO + NAL + CIP + SOX	$bla_{CTX-M-27}$
ECPDS749	4	AMP + AXO + TET + CHL + SOX	$bla_{CTX-M-14}$

[a]These isolates did not possess any SHV, TEM, CTX-M, or CMY-2 type β-lactamases.

TABLE 5 Phenotypic and genotypic characteristics of CTX-M and PMQR producing *E coli* isolated from the urine of dogs

Isolate	Phenotype	Genotype	ST
ECPDS202	AMP + AXO + XNL + NAL + TET + SOX + SXT	$bla_{CTX-M-15}$	ST10
ECPDS218	AMP + AUG + FOX + AXO + XNL + NAL + CIP + TET + SOX	*aac(6′)- Ib-cr*	ST38
ECPDS236	AMP + AXO + XNL	$bla_{CTX-M-15}$	ST12
ECPDS318	AMP + AXO + XNL + NAL + GEN + TET + CHL + SOX + SXT + AZI	$bla_{CTX-M-27}$	ST131
ECPDS494	AMP + AXO + XNL + NAL + CIP + TET + CHL + AZI	$bla_{CTX-M-14}$	ST648
ECPDS552	AMP + AXO + NAL + CIP + TET + SOX + SXT + AZI	$bla_{CTX-M-27}$	ST1193
ECPDS698	AMP + AXO + NAL + CIP + SOX	$bla_{CTX-M-27}$	ST38
ECPDS749	AMP + AXO + TET + CHL + SOX	$bla_{CTX-M-14}$	ST117

3.4 | Typing of ESBL- and PMQR-producing isolates

MLST was performed on 7 ESBL producers, as well as the single isolate harboring the *aac(6′)-Ib-cr* gene (Table 5). Two isolates belonged to ST38; 1 possessed an *aac(6′)-Ib-cr* gene, while the other produced the ESBL $bla_{CTX-M-27}$. The remaining isolates belonged to distinct sequence types including ST10, ST12, ST117, ST131, ST648, and ST1193. The isolates producing $bla_{CTX-M-14}$ belonged to ST117 and ST648. The isolates harboring $bla_{CTX-M-15}$ belonged to ST10 and

ST12. The 2 remaining $bla_{CTX\text{-}M\text{-}27}$ producers belonged to ST131 and ST1193.

4 | DISCUSSION

The most recent treatment guidelines from ISCAID recommend amoxicillin (±clavulanate) or SXT for the empirical treatment of uncomplicated cystitis in dogs.[8] This study suggests that *E coli* isolated from the urine of dogs in our region remain quite susceptible to first-line therapies.[8]

Resistance to ampicillin was most common (~15%), while fluoroquinolone susceptibility remained high (~98%) in this isolate population (n = 516). This is in contrast to AMR trends observed in *E coli* of human urine origin.[23-25] In fact, a lack of efficacy coupled with the alarming rates of resistance has caused the Infectious Diseases Society of America (IDSA) to remove amoxicillin from their treatment guidelines for uncomplicated cystitis in women, while fluoroquinolones are reserved for important uses other than uncomplicated cystitis.[26] In general, clinical guidelines recommend that local resistance rates exceeding 10% indicate an emerging resistance problem.[7] Although the frequency of ampicillin resistance identified is concerning, the high urinary concentrations reached by amoxicillin (the oral aminopenicillin of choice) far exceed the clinical breakpoint used to interpret susceptibility test results in dogs.[8]

Two previous studies have described the antimicrobial susceptibility of *E coli* from the urine of dogs in this region. The most recent was a single year (2013-2014) of passive surveillance preceding the present study. In this investigation, a high proportion of pan-susceptible isolates (79.6%, 90/113) were identified, ampicillin resistance was the most common (8.8%, 10/113 of isolates), with low levels of quinolone resistance with just 7% (8/113) of isolates exhibiting resistance to nalidixic acid and <1% (1/113) displaying ciprofloxacin resistance. Single $bla_{CMY\text{-}2}$-type AmpC β-lactamase producers were identified and no ESBLs were detected.[12] An earlier study published in 2008 reported no change in the number of antimicrobials to which *E coli* from nonrecurrent infections were resistant from 2002 to 2007.[27]

We report the emergence of important AMR mechanisms in this region. In contrast to our previous findings, we report the identification of ESBLs of the CTX-M type, the PMQR determinant *aac(6′)-Ib-cr*, and the pandemic clone *E coli* ST131. CTX-M-type ESBLs are a globally disseminated and highly transmissible form of third-generation cephalosporin resistance that is commonly reported among *E coli* causing community-acquired cystitis in humans.[11] CTX-M type enzymes are present in *E coli* isolated from canine urine in Australia, Europe, Asia, and the United States.[28-35] Coresistance to fluoroquinolones is a well-documented phenomenon among ESBL-producing *E coli* isolated from urine in human medicine.[36] However, of the fluoroquinolone-resistant ESBL producers reported here, none possessed the allele $bla_{CTX\text{-}M\text{-}15}$, which is detected most frequently among isolates displaying fluoroquinolone resistance in the human literature.[36]

The PMQR determinants *qnr*, *aac(6′)-Ib-cr*, and *qep*A have been thoroughly investigated in the human literature.[37] The *aac(6′)-Ib-cr* gene is the most widely disseminated PMQR determinant and has been found in *E coli* from healthy and diseased companion animals in Europe, Asia and the United States.[30,38,39] Here, we describe Aac(6′)-Ib-cr producing *E coli* from a dog in Canada. *Escherichia coli* harboring *aac(6′)-Ib-cr* have been frequently detected in isolates from community-acquired UTIs. In people, these strains frequently also possess CTX-M type ESBLs.[36,37] In fact, here we identify *E coli* ST131 in a clinical isolate of companion animal origin in Canada. This globally prevalent clone is one such example where fluoroquinolone resistance and ESBL production came together to produce a highly pathogenic and MDR urinary *E. coli* strain.[40]

Epidemiologically, AMR spreads either by the clonal dissemination of a single resistant strain or by nonclonal mechanisms including the horizontal transfer of resistance determinants in an otherwise heterogeneous population. Although the high prevalence of ST131 among community-acquired UTIs in the human literature is one such example of clonal spread, the results of the current study more closely reflect the second mechanism.[40] Not only did the 7 ESBL-producing isolates identified exhibit distinct phenotypic resistance profiles, but each was a unique sequence type. These findings suggest that the population of urinary *E coli* in our region is genetically heterogeneous and that resistance has most likely emerged by the horizontal dissemination of resistance genes on mobile genetic elements, rather than elaboration of a single successful resistant clone. Among companion animal *E coli*, there is a lack of evidence for a concrete linkage between a specific gene and a certain sequence type. Furthermore, our results are consistent with previous studies which have found that ESBL-producing isolates belong to a variety of sequence types in contrast to the more homogeneous strains identified in people.[28,41,42]

The current study applied a rigorous inclusion criteria and gold standard microbiological methods, including quantitative susceptibility testing and molecular epidemiological techniques (MLST) to describe AMR among *E coli* from canine urine in our region. This is in contrast to previous surveillance studies, which relied on convenience sampling of susceptibility data, or isolates, submitted to diagnostic laboratories. Although this study design is convenient, passive surveillance does not allow for the prospective collection of clinical data on each case. This precluded analysis of associations among previous antimicrobial treatment, comorbidities, and clinical outcome as possible risk factors for infection with a resistant organism. For example, a study describing the resistance phenotypes of isolates collected from recurrent UTIs in the same animal would allow for the true association between previous antimicrobial therapy and infection with MDR pathogens to be more completely described. To this end, there is an urgent need to improve the quality of laboratory information systems to improve both the consistency and accessibility of meta data to complete such studies.

The robust methodology applied here provides a strong example of disease-specific AMR surveillance and its application to pathogens of companion animals. These findings offer invaluable information about region-specific AMR trends for companion animal veterinarians in informing empirical treatment of UTI, while also establishing a baseline for future surveillance activities. Although the *E coli* from canine

urine examined in this study remain susceptible, the detection of broad-spectrum beta-lactamases, PMQR, and >10% ampicillin resistance highlights the importance of continued monitoring.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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