Occurrence and potential transmission of extended-spectrum beta-lactamase-producing extraintestinal pathogenic and enteropathogenic *Escherichia coli* in domestic dog faeces from Minnesota

Timothy J. Johnson1 | Joseph R. Armstrong2 | Brian Johnston3 | Irene Merino-Velasco4 | Ivana Jamborova5 | Randall S. Singer1 | James R. Johnson3 | Jeff B. Bender6

1Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA
2Center for Agriculture, Food, and Natural Resources, University of Minnesota Extension, Saint Paul, Minnesota, USA
3Minneapolis Veterans Affairs Medical Center, Minneapolis, MN, USA, and Department of Medicine, University of Minnesota, Minneapolis, Minnesota, USA
4Hospital Universitario Ramón y Cajal-IRYCS, Madrid, Spain
5University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
6Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA

**Abstract**

Interactions between humans and pets are increasingly valued in western countries, leading to more extensive contact between humans and their pets within households. Although the magnitude of the risk of transfer of *Escherichia coli* between humans and their companion animals is undefined, that such transmission occurs has been established and warrants attention. This study examined 186 fresh faecal samples from companion dogs visiting 22 municipal dog parks in the Minneapolis/Saint Paul metropolitan area, Minnesota, USA. Samples were processed to isolate 3rd-generation cephalosporin-resistant *E. coli*, which were further characterized using PCR-based virulence genotyping, antimicrobial susceptibility profiling and whole-genome sequencing. Of the 186 faecal samples, 29% yielded cephalosporin-resistant *E. coli*, and 2.2% yielded extended-spectrum beta-lactamase producers. Co-resistance to sulfonamides was typical (77.3% of isolates), and multidrug resistance (i.e. to ≥3 antimicrobial classes), including to combinations of tetracyclines, phenicols, quinolones and aminoglycosides, was substantial (18.9% of isolates). Identified beta-lactamase genes included *bla*<sub>CMY-2</sub>, *bla*<sub>TEM-1B</sub>, *bla*<sub>TEM-1</sub>, *bla*<sub>CTX-M-24</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-1</sub>. Genome sequencing of 14 isolates identified genes typical of extraintestinal pathogenic *E. coli* or enteropathogenic *E. coli*. In three instances, closely related isolates were recovered from different dogs, within either the same park—suggesting transfer of *E. coli* between dogs within the park—or different parks—suggesting that dogs may be...
Domestic dogs (Canis familiaris) are integral parts of households of many humans across the world. Increasingly, domestic dogs share close contact with human household members, and in urban settings, often congregate at designated dog parks where they are allowed to roam grassy landscapes and interact with other dogs from different households (Madec et al., 2017; PRNewswire T. H. P., 2015).

Numerous studies have investigated the carriage of Escherichia coli by healthy companion dogs, many of which address the faecal carriage of cephalosporin-resistant and extended-spectrum beta-lactamase (ESBL)-producing E. coli from healthy companion dogs. In these studies, the proportion of positive faecal or rectal swabs ranged from 4% to 45% for cephalosporin-resistant E. coli (Aslantas & Yilmaz, 2017; Carvalho et al., 2016; Damborg et al., 2015; Hordijk et al., 2013; Ortega-Paredes et al., 2019; Rocha-Gracia et al., 2015; Schmidt et al., 2015; Umeda et al., 2019), and the proportion of faecal samples positive for ESBL-producing E. coli ranged from 2% to 12% (Belas et al., 2014; Karkaba et al., 2019; Rocha-Gracia et al., 2015; Wedley et al., 2017; Yousfi et al., 2016). Very few such studies have been conducted in the United States (US). In one US study, 6/61 (9.8%) faecal samples from healthy dogs yielded cephalosporin-resistant E. coli (Stenske et al., 2009), and in another, 0/15 dog faecal isolates from dog parks yielded ceftriaxone-resistant E. coli (Ahmed et al., 2015).

The purpose of this study was to examine faecal samples from dogs visiting dog parks in the Minneapolis/Saint Paul metropolitan area in Minnesota, the United States, for the presence of cephalosporin-resistant and ESBL-producing E. coli. The goals were to determine whether such isolates are circulating among dogs and to determine whether shared clones exist between dogs frequencing the same or different dog parks.

2 | MATERIALS AND METHODS

From July to August 2013, research staff visited 22 different dog parks in the Minneapolis/Saint Paul metropolitan area (Armstrong et al., 2015). From these parks, collectively, 186 fresh faecal samples (average 8.5 samples per park) were collected aseptically into sterile faecal collection containers (Fisher Scientific) using provided sterile plastic spatulas and gloves. Samples were labelled as to specific dog park, dog within that park and (if multiple dogs per owner) owner. All samples came from different dogs and, with rare exceptions, different owners.

Samples were processed promptly for cefotaxime-resistant E. coli by placing 1 g of faecal material into 10-ml Luria-Bertani (LB) broth (Becton-Dickinson) containing 1-ug/mL cefotaxime and incubating overnight with shaking at 37°C. The same sample was also placed in 10-ml LB broth without antibiotic overnight to confirm growth of total E. coli. The following day, a 1-μl loop of the overnight growth was streaked onto MacConkey agar (Becton-Dickinson) with 1-μg/ml cefotaxime, and a representative suspect E. coli colony from each plate was selected. MacConkey agar with no antibiotic was included to confirm E. coli recovery from each sample. ChromAgar and an E. coli-specific polymerase chain reaction (PCR) were then used to confirm that colonies were indeed E. coli (Walk et al., 2009). Presumptive cefotaxime-resistant isolates were stored in 20% glycerol until further use.

Presumptive cefotaxime-resistant isolates underwent antimicrobial susceptibility testing using the National Antimicrobial Resistance Monitoring System panel CMV2AGNF by Trek Diagnostics according to Food and Drug Administration, US Department of Agriculture, and Clinical Laboratory Standards Institute recommendations (CLSI; CLSI, 2017). This plate was designed for the testing of veterinary isolates (McDermott et al., 2016) but do not necessarily represent canine-specific breakpoints and allows determination of broth microdilution minimum inhibitory concentrations (MIC) for 15 antimicrobials (drug name abbreviation; resistance breakpoint used): amoxicillin/clavulanic acid (AUG; ≥32/16 μg/ml), ampicillin (AMP; ≥32 μg/ml), azithromycin (AZI; ≥32 μg/ml), cefoxitin (FOX; ≥32 μg/ml), ceftiofur (TIO; ≥8 μg/ml), ceftriaxone (AXO; ≥4 μg/ml), chloramphenicol (CHL; ≥8 μg/ml), ciprofloxacin (CIP; ≥0.5 μg/ml), and gentamicin (GEN; ≥1 μg/ml).
spectively. 

FimTyper (Roer et al., 2017) and SerotypeFinder (Joensen et al., 2020) were used to identify fimH allele and predicted serotypes. They also contained a variety of plasmid replicon types, including IncA/C2 (now separated into IncA and IncC), IncFIA, IncFIB, IncFIC, IncFII, IncI1, IncI2, IncQ1 and IncX1. Of the 186 faecal samples from 22 municipal dog parks, 100% (186/186) yielded E. coli after incubation in antibiotic-free LB broth, vs. 29% (54/186) after incubation in cephalosporin-supplemented (1 μg/ml) LB broth, and 2.2% (4/186) yielded ESBL-producing E. coli according to CLSI guidelines (CLSI, 2017).

Cefotaxime-resistant isolates underwent multiplex PCR-based phylotyping (Clermont et al., 2000) and extended virulence genotyping for 31 putative or proven virulence genes associated with extraintestinal pathogenic E. coli (ExPEC) (Johnson et al., 2015). Presumptive ExPEC status was assigned based on presence of ≥2 of five established indicator genes (Johnson et al., 2003).

Whole-genome sequencing was performed for 14 isolates total, including those that displayed an ESBL phenotype (n = 4) or reduced susceptibility to ≥6 of the 15 antimicrobials in the CMV2AGNF MIC panel (n = 10). The purpose of this approach was to further study ESBL producers and those with cefotaxime resistance in the presence of additional resistance phenotypes. DNA extractions were performed using overnight growths in LB broth of a single inoculated colony using the Qiagen DNeasy kit following manufacturer instructions. Genomic DNA libraries were created using Nextera XT library preparation kits and Nextera XT index kit v2 (Illumina), and sequencing was performed using 2×250 bp dual-index runs on an Illumina MiSeq at the University of Minnesota Mid-Central Research and Outreach Center. Targeted sequencing coverage was 40–50x.

Following assembly with SPAdes (Bankevich et al., 2012), resistance genes and plasmid replicons were identified using Resfinder (Zankari et al., 2012) and PlasmidFinder (Carattoli et al., 2014), respectively. FimTyper (Roer et al., 2017) and SerotypeFinder (Joensen et al., 2015) were used to determine fimH allele and predicted serotype, respectively. VirulenceFinder (Kleinheinz et al., 2014) was used for identification of E. coli virulence genes. A custom database of 46 additional genes associated human and avian ExPEC (https://doi.org/10.6084/m9.figshare.11337278.v1) was also used to identify additional virulence-associated genes using ABRicate (https://github.com/tseemann/abricate). For genome-sequenced isolates, a previously established definition for intestinal E. coli pathotypes was used (Bugarel et al., 2011). ClustVis (Metsalu & Vilo, 2015) was used to display virulence and antimicrobial susceptibility data in heatmap format.

The 7-gene Achtman multilocus sequence typing (MLST) database (Larsen et al., 2012) was used to assign a sequence type (ST) to each isolate. Clonality between isolates was defined as isolates differing by ≤40 whole-genome single nucleotide polymorphisms (SNPs), following previous guidance for such definitions (Salipante et al., 2015).

The methods and protocols for this study were reviewed by the University of Minnesota Institutional Animal Care and Use Committee and determined to be exempt from a need for ethical approval.

Raw sequencing data from this project are deposited in the NCBI short read archive under BioProject number PRJNA593904.

3 | RESULTS AND DISCUSSION

Of the 186 faecal samples from 22 municipal dog parks, 100% (186/186) yielded E. coli after incubation in antibiotic-free LB broth, vs. 29% (54/186) after incubation in cephalosporin-supplemented (1 μg/ml) LB broth, and 2.2% (4/186) yielded ESBL-producing E. coli according to disk diffusion. Broth microdilution testing showed that most of the 54 presumptive cefotaxime-resistant isolates were co-resistant to other beta-lactams, including ampicillin (100%), ceftriaxone (94%), cefotaxime (83%), ceftriaxone (80%) and amoxicillin/clavulanic acid (59%; Figure 1). As for non-beta-lactams, resistance was variably prevalent also to sulfisoxazole (76%), tetracycline (15%), nalidixic acid (11%) and streptomycin (11%); 19% of the 54 cefotaxime-resistant isolates exhibited multidrug resistance.

All 54 cefotaxime-resistant isolates possessed fimH and uidA using PCR, confirming they were E. coli. According to PCR-based profiling, the most frequent ExPEC-associated virulence-associated genes among these isolates were traT (50%), fyuA (39%), chromosomal ompT (37%), iroN (30%) and malX (26%); and 11% (6/54) isolates qualified molecularly as ExPEC.

Whole-genome sequencing was performed on the 4 isolates with an ESBL phenotype and 10 additional isolates displaying resistance to ≥6 of the tested drugs (Table 1). Sequence analysis showed that these isolates contained diverse beta-lactamase genes, including blα_{TEM-1} (n = 2), blα_{TEM-15} (n = 1), blα_{CTX-M-15} (n = 1), blα_{CTX-M-24} (n = 2), blα_{CTX-M-4} (n = 1) and blα_{OXA-1} (n = 1). Some isolates possessed co-occurring resistance genes encoding aminoglycoside resistance (strAB, aadA1, aadA2, ahpC2-3)-, lacA (3′)-IId and/or acs(6′)-IId-cr, macrolide resistance (mph[A]), phenicol resistance (floR, catA1 or catB3), sulphonamide resistance (sul1 and/or sul2), tetracycline resistance (tet(A) or tet(B)) and trimethoprim resistance (dfra3). Isolates belonged to diverse STs and exhibited diverse fimH alleles and predicted serotypes. They also contained a variety of plasmid replicon types, including IncA/C2 (now separated into IncA and IncC), IncFIA, IncFIB, IncFIC, IncFII, IncI1, IncI2, IncQ1 and IncX1.

Several genome-sequenced isolates possessed ExPEC-associated characteristics. For example, two (non-ESBL-producing) isolates from different dog parks (isolates DP8-5 and DP25-3) represented ST12/serotype O4:H5, which previously was found to
overlap between canine and human urinary tract infection isolates in the United States (Johnson et al., 2001). These isolates possessed genes corresponding with the yersiniabactin siderophore system (fyuA and irp2), the salmochelin siderophore system (iroBCDEN), pyelonephritis-associated pili (papC) and ColV or ColBM plasmids (cvaAB, cbi, cmi and cma). They differed by >6500 core genome SNPs, so represented distinct strains.

Three other (non-ESBL-producing) isolates, from three different dogs/owners in the same dog park, represented ST372 (serotype O15:H31), which is a sequence type previously linked to human ExPEC and a predominant strain in canine ExPEC infections (Flament-Simon et al., 2020; Kidsley et al., 2020). These isolates possessed identical resistance profiles, resistance genotypes and virulence genotypes, including multiple ExPEC-associated virulence genes.
| Isolate  | 7-gene ST  | fimH allele | Predicted serotype | Pathotype | Virulence-associated genes | Resistance profile | Resistance genes | Plasmid replicons |
|----------|------------|-------------|-------------------|-----------|---------------------------|-------------------|-----------------|------------------|
| DP8-5    | ST12       | H204        | O4:H5             | ExPEC     | cbi, cma, cmi, cvaAB, fyuA, gad, hlyF, iroBCDEN, irp2, iss, mchB, mchC, mchF, mcmA, ompTp, papC, sitABCDE, tia, vat | FOX, TET, AXO, AUG2, XNL, FIS, SXT, AMP, STR, CAZ | strA, strB, aadA1, bladTEM-1, bladCMY-2, sul1, sul2, tetA, dfrA1 | IncFIB (AP001918), IncFII, IncQ1 |
| DP8-9    | ST88       | H27         | O8:H19            | ExPEC     | cvaABC, cvi, etsABC, fyuA, hlyF, iroBCDEN, irp2, iss, iucABCDe, iutA, lpfA, mchF, ompTp, sitABCDE | FOX, CTX, CAZ, TET, AXO, AUG2, XNL, FIS, KAN, AMP, STR, AMP | strA, strB, aph(3’)-Ia, bladTEM-1, bladCMY-2, sul2, tetA | IncFIB (AP001918), IncFII, IncI2, IncQ1 |
| DP14-7   | ST297      | H1380       | O169:H8           | None      | cib, cibl, gad, lpfA, sitABCDE | FOX, AXO, AUG2, XNL, FIS, AMP | bladCMY-2 | IncI1, IncI2 |
| DP18-2A  | ST517      | H32         | O71:H19           | EPEC      | eae, espA, espF, gad, lpfA, nleA, nleB, nleC, perA, tir | FOX, FIS, AMP |            |                  |
| DP18-2B  | ST372      | H9          | O15:H31           | ExPEC     | cnf1, cvaAB, fyuA, gad, iueAB, iroBCDEN, irp2, iss, mchB, mchC, mchF, mcmA, papC, sitABCDE, vat | FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP | bladCMY-2 | IncI1 |
| DP18-4   | ST372      | H9          | O15:H31           | ExPEC     | cnf1, cvaAB, fyuA, gad, iueAB, iroBCDEN, irp2, iss, mchB, mchC, mchF, mcmA, papC, sitABCDE, vat | FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP | bladCMY-2 | IncI1 |
| DP18-3   | ST372      | H9          | O15:H31           | ExPEC     | cnf1, cvaAB, fyuA, gad, iueAB, iroBCDEN, irp2, iss, mchB, mchC, mchF, mcmA, papC, sitABCDE, vat | FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP | bladCMY-2 | IncI1 |
| DP21-3   | ST10       | NT          | O26:H36           | None      | aatA, aec35-36-37, astA, capU, gad, iore | CTX, CAZ, CHL, TET, AXO, NAL, XNL, FIS, SXT, AMP, STR | strA, strB, bladTEM-18, bladCMY-15, catA1, sul1, sul2, tetB, dfrA7 | IncQ1 |
| DP23-1   | ST10       | H24         | O157:H16          | EPEC      | cebL, eae, eitABCDe, espA, espF, gad, nleB, nleC, sepA, tir | CTX, AXO, XNL, FIS, AMP | bladCMX-24 | IncFII |
| DP24-5   | ST155      | H366        | O:-H9             | ExPEC     | aatA, astA, cib, cibl, cvaABC, cvi, eitABCDe, etsABC, fyuA, gad, iroBCDEN, irp2, iss, iucABCDe, iutA, lpfA, mchF, ompTp, sitABCDE, tsh | FOX, CTX, AXO, XNL, FIS, AMP | bladCMX-1, sul2 | IncFIA/FIB/FIC, IncI1 |
| DP25-1   | ST38       | H65         | O7:H15            | None      | air, eliA, gad, iss, sitABCDE | FOX, CAZ, CHL, TET, AXO, AUG2, GEN, XNL, FIS, SXT, AMP, STR | strA, strB, aac(3)-IId, aadA5, bladTEM-18, bladCMY-2, floR, sul1, sul2, tetA, dfrA17 | IncFII, IncAC2 |
| DP25-3   | ST12       | H27         | O4:H5             | ExPEC     | cvaAB, fyuA, gad, iroBCDEN, irp2, iss, mchB, mchC, mchF, mcmA, papC, sitABCDE, vat | FOX, CHL, TET, AXO, AUG2, XNL, FIS, SXT, AMP, STR, AMP | strA, strB, aadA2, bladTEM-18, bladCMY-2, floR, sul1, sul2, tetA, dfrA12 | IncAC2, IncX1 |
| DP26-1   | ST10       | H24         | O157:H16          | EPEC      | cebL, eae, eitABCDe, espA, espF, nleB, nleC, sepA, tccP, tir | CTX, AXO, XNL, FIS, AMP | bladCMX-24 | IncFIB/FII |
| DP26-2A  | ST224      | H61         | O8:H23            | None      | gad, lpfA | FOX, CAZ, TET, AXO, AUG2, CIP, NAL, FIS, AMP | aac(6')Ib-cr, bladOX-1, bladCMY-2, bladTEM-18, catB3, tetB | IncFIA/FII/FIB |
(cnf1, vat, cvaAB, fyuA, irp2, ibeA, papC, iroBCDEN and sitABCD); cnf1 is a defining trait of necrotoxicogenic E. coli (DebRoy & Maddox, 2001). They differed by <40 core genome SNPs and therefore represented highly similar clones. Because many dogs visited these same dog parks repeatedly (survey data not shown), the observed commonality could indicate strain-sharing events that occurred during prior direct or indirect contact.

By contrast, 3 of the 14 genome-sequenced isolates (DP18-2A, DP23-1 and DP26-1) possessed key traits of asymptomatic enteropathogenic E. coli (EPEC), including eae, espABF, nleABC, perA, sepA and tir (Deng et al., 2004). Two of these isolates (both, ESBL-producers) represented ST10/serotype O157:H16, and one (a non-ESBL-producer) represented ST517/serotype O71:H19. Both of these serotypes are associated with EPEC (Blanco Crivelli et al., 2018; Feng et al., 2012), and intimin-producing O157:H16 strains have been previously found in dogs (Bentancor et al., 2010). The O157:H16 isolates differed by <170 core genome SNPs, so represent closely related but non-identical strains from different dogs at different dog parks, while the O71:H19 isolate was genetically distinct (>1000 SNPs different) from the O157:H16 isolates and was found at yet another dog park.

4 | CONCLUSIONS

Although quantifiable risk has not yet been established for the transmission of commensal bacteria such as E. coli between humans and companion animals (Madec et al., 2017), mounting evidence indicates that such transmissions do occur and must be considered. This study’s findings suggest that dog parks present an opportunity for drug-resistant and potentially pathogenic E. coli to be spread between visiting animals. Consequently, both the parks and the dogs themselves may pose some risk to human owners through clone sharing and dissemination, possibly followed by subsequent within-household transmission. The identification of ExPEC and EPEC isolates harbouring drug resistance in dog faeces, with evidence suggesting transmission between dogs within the same dog park, highlights the fact that such transmission events may pose a risk with regard to both pathogenic potential and further dissemination of the antibiotic resistance gene pool within a community. This study was limited by small sample size, limited geographic area studied, use of only one colony per isolate, and bias for the selection and further characterization of only cepotaxime-resistant E. coli. This study was also limited by the use of a food production-oriented susceptibility panel, which uses different antimicrobials and breakpoints than companion animal susceptibility panels. However, these findings suggest that dog park visits may serve as an opportunity for acquisition of MDR E. coli with pathogenic potential, and public awareness of the need for hygienic practices in these parks is warranted.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NCBI Short Read Archive at https://www.ncbi.nlm.nih.gov/sra.

ORCID

Timothy J. Johnson https://orcid.org/0000-0001-7556-9347
Randall S. Singer https://orcid.org/0000-0002-5461-9330

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