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SHORT COMMUNICATION

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

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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 $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-24}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$. 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

pre-disposed to carry certain *E. coli* types, such as those from serogroups O4, O71 and O157. This study adds to the existing evidence that companion dogs can harbour and share antimicrobial-resistant *E. coli* with presumed intestinal or extraintestinal pathogenic potential.

KEYWORDS
canine, cephalosporin, *Escherichia coli*, faecal

1 | INTRODUCTION

Domestic dogs (*Canis familiaris*) are integral parts of the 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 frequenting the same or different dog parks.

Impacts

- Healthy dogs within the Twin Cities metropolitan area harbour cephalosporin-resistant *Escherichia coli* in their normal faecal flora.
- Some cephalosporin-resistant isolates from dog faeces harboured traits typical of enteropathogenic *E. coli* (EPEC) or extraintestinal pathogenic *E. coli* (ExPEC).
- There was evidence of sharing of *E. coli* clones between different dogs via community 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;

≥32 μg/ml), ciprofloxacin (CIP; ≥ 1 μg/ml), gentamicin (GEN; ≥ 16 μg/ml), kanamycin (KAN; ≥64 μg/ml), nalidixic acid (NAL; ≥32 μg/ml), streptomycin (STR; ≥32 μg/ml), sulfisoxazole (FIS; ≥512 μg/ml), trimethoprim/sulfamethoxazole (SXT; ≥4/76 μg/ml) and tetracycline (TET; ≥16 μg/ml). Inoculation of panels was done per the manufacturer's instructions. Intermediate isolates were not differentiated from susceptible isolates for this analysis. CLSI-specified control strains of *E. coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* were used to validate each run. Multidrug resistance was defined as resistance to at least one agent in ≥3 different antimicrobial classes.

Additionally, all cefotaxime-resistant isolates were screened for ESBL production by first examining disk diffusion susceptibility to cefoxitin (FOX; 30 μg), cefotaxime (CTX; 30 μg) and ceftazidime (CAZ; 30 μg) on commercial disks (Becton-Dickinson). Isolates with reduced susceptibility to CTX and/or CAZ were then assessed for the presence of ESBLs using a combination of the double-disk synergy test for CTX and CAZ with clavulanic acid (Becton-Dickinson) 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 40–50×.

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 cefotaxime-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%), cefoxitin (83%), ceftiofur (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 $bla_{\text{CMY-2}}$ ($n = 9$), $bla_{\text{TEM-1}}$ ($n = 2$), $bla_{\text{TEM-1B}}$ ($n = 3$), $bla_{\text{CTX-M-15}}$ ($n = 1$), $bla_{\text{CTX-M-24}}$ ($n = 2$), $bla_{\text{CTX-M-1}}$ ($n = 1$) and $bla_{\text{OXA-1}}$ ($n = 1$). Some isolates possessed co-occurring resistance genes encoding aminoglycoside resistance (*strAB*, *aadA1*, *aadA2*, *aph(3′)-Ia*, *aac(3)-IId* and/or *aac(6′)Ib-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 (*dfrA1*, *dfrA7*, *dfrA12* or *dfrA17*). 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

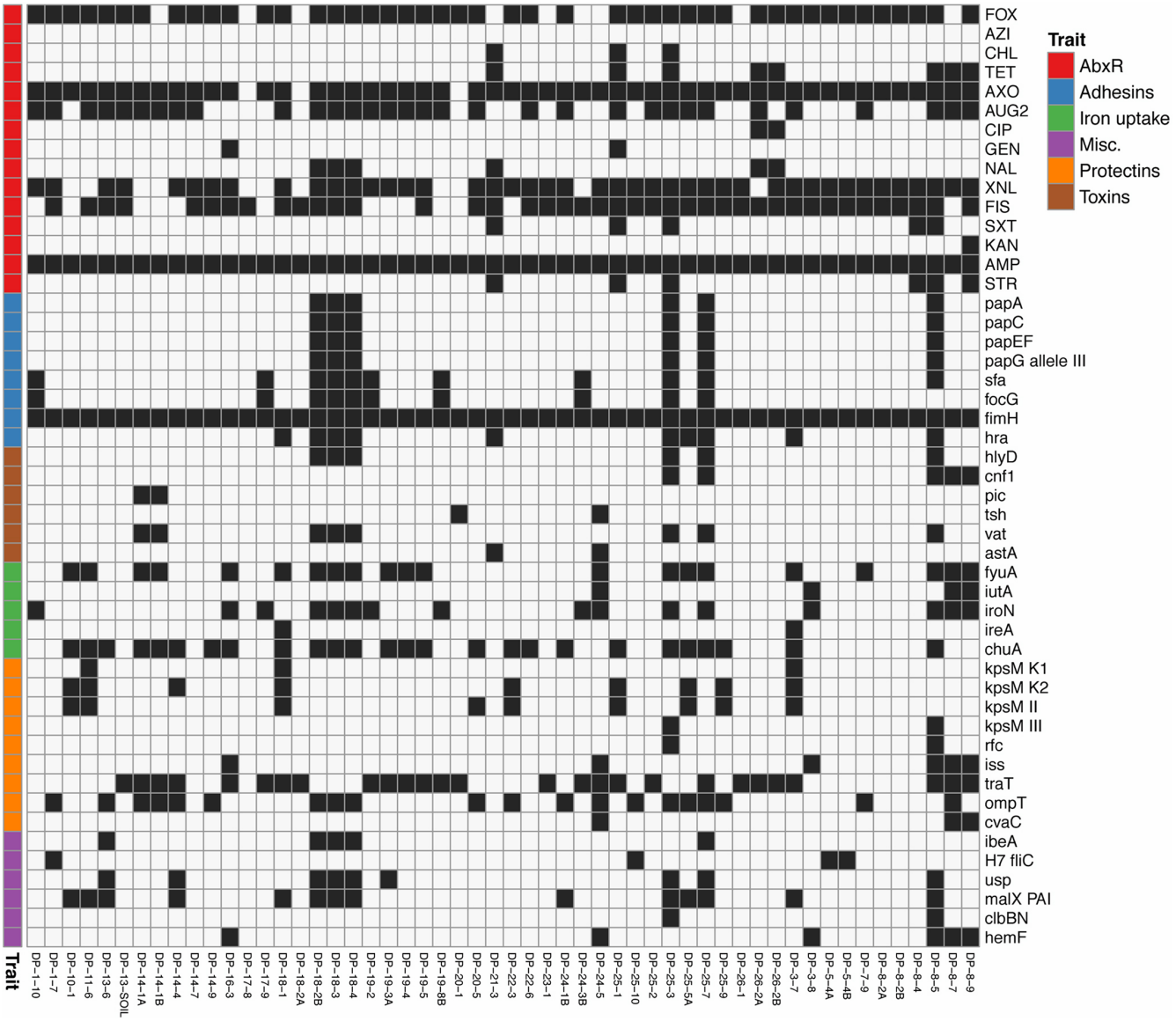

FIGURE 1 Results of antimicrobial susceptibility testing and PCR-based virulence genotyping for 54 cephalosporin-resistant *Escherichia coli* from dog faeces. Black boxes indicate resistance to an antimicrobial or a positive PCR reaction. See methods section for antibiotic and virulence gene abbreviations. For virulence-associated genes, *pap* = pyelonephritis-associated pili; *sfa* = S fimbrae; *focG* = F1C fimbriae; *fimH* = type 1 fimbriae; *hra* = heat-resistant agglutinin; *hlyD* = haemolysin; *cnf1* = cytotoxic necrotizing factor; *pic* = serine protease; *tsh* = temperature-sensitive hemagglutinin; *vat* = vacuolating autotransporter; *astA* = EAST1 enterotoxin; *fyuA* = ferric yersiniabactin uptake; *iutA* = aerobactin siderophore system; *iroN* = salmochelin siderophore system; *ireA* = iron-regulated element; *chuA* = haem-binding outer membrane; *kpsM* K1 = K1 group II capsule; *kpsM* K2 = K2 group II capsule; *kpsM* II = group II capsule; *kpsM* III = group III capsule; *rfc* = O4 LPS synthesis; *iss* = increased serum survival; *traT* = surface exclusion; *ompT* = outer membrane protease; *cvaC* = ColV microcin operon; *ibeA* = invasion of brain epithelium; H7 *fliC* = H7 flagellin variant; *usp* = uropathogenic-specific microcin; *malX* = pathogenicity-associated island marker; *cldBN* = hybrid peptide-polyketide synthase; *hemF* = non-ribosomal synthetase. Antibiotics included amoxicillin/clavulanic acid (AUG), ampicillin (AMP), azithromycin (AZI), cefoxitin (FOX), ceftiofur (TIO), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), trimethoprim/sulfamethoxazole (SXT) and tetracycline (TET)

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

TABLE 1 Traits of whole-genome-sequenced, cephalosporin-resistant *Escherichia coli* isolates from dog parks

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*, *sitABCD*, *tia*, *vat*	FOX, TET, AXO, AUG2, XNL, FIS, SXT, AMP, STR, CAZ	*strA*, *strB*, *aadA1*, bla_{TEM-1}, bla_{CMY-2}, *sul1*, *sul2*, *tetA*, *dfrA1*	IncFIB (AP001918), IncFII, IncQ1
DP8-9	ST88	*H27*	O8:H19	ExPEC	*cvaABC*, *cvi*, *etsABC*, *fyuA*, *hlyF*, *iroBCDEN*, *irp2*, *iss*, *iucABCD*,*iutA*, *lpfA*, *mchF*, *ompTp*, *sitABCD*	FOX, CTX, CAZ, TET, AXO, AUG2, XNL, FIS, KAN, AMP, STR	*strA*, *strB*, *aph(3′)-Ia*, bla_{TEM-1}, bla_{CMY-2}, *sul2*, *tetA*	IncFIB (AP001918), IncFII, IncI2, IncQ1
DP14-7	ST297	*H1380*	O169:H8	None	*cib*, *cibI*, *gad*, *lpfA*, *sitABCD*	FOX, AXO, AUG2, XNL, FIS, AMP	bla_{CMY-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 (NTEC)	*cnf1*, *cvaAB*, *fyuA*, *gad*, *ibeA*, *iroBCDEN*, *irp2*, *iss*, *mchB*, *mchC*, *mchF*, *mcmA*, *papC*, *sitABCD*, *vat*	FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP	bla_{CMY-2}	IncI1
DP18-4	ST372	*H9*	O15:H31	ExPEC (NTEC)	*cnf1*, *cvaAB*, *fyuA*, *gad*, *ibeA*, *iroBCDEN*, *irp2*, *iss*, *mchB*, *mchC*, *mchF*, *mcmA*, *papC*, *sitABCD*, *vat*	FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP	bla_{CMY-2}	IncI1
DP18-3	ST372	*H9*	O15:H31	ExPEC (NTEC)	*cnf1*, *cvaAB*, *fyuA*, *gad*, *ibeA*, *iroBCDEN*, *irp2*, *iss*, *mchB*, *mchC*, *mchF*, *mcmA*, *papC*, *sitABCD*, *vat*	FOX, CTX, AXO, AUG2, NAL, XNL, FIS, AMP	bla_{CMY-2}	IncI1
DP21-3	ST10	NT	O26:H36	None	*aatA*, *aec35-36-37*, *astA*, *capU*, *gad*, *iroDE*	CTX, CAZ, CHL, TET, AXO, NAL, XNL, FIS, SXT, AMP, STR	*strA*, *strB*, bla_{TEM-1B}, $bla_{CTX-M-15}$, *catA1*, *sul2*, *tetB*, *dfrA7*	IncQ1
DP23-1	ST10	*H24*	O157:H16	EPEC	*celb*, *eae*, *eitABCD*, *espA*, *espB*, *espF*, *gad*, *nleB*, *nleC*, *sepA*, *tir*	CTX, AXO, XNL, FIS, AMP	$bla_{CTX-M-24}$	IncFII
DP24-5	ST155	*H366*	O-:H9	ExPEC	*aatA*, *astA*, *cib*, *cibI*, *cvaABC*, *cvi*, *eitABCD*, *etsABC*, *fyuA*, *gad*, *iroBCDEN*, *irp2*, *iss*, *iucABCD*, *iutA*, *lpfA*, *mchF*, *ompTp*, *sitABCD*, *tsh*	FOX, CTX, AXO, XNL, FIS, AMP	$bla_{CTX-M-1}$, *sul2*	IncFIA/FIB/FIC, IncI1
DP25-1	ST38	*H65*	O7:H15	None	*air*, *eilA*, *gad*, *iss*, *sitABCD*	FOX, CAZ, CHL, TET, AXO, AUG2, GEN, XNL, FIS, SXT, AMP, STR	*strA*, *strB*, *aac(3)-IId*, *aadA5*, bla_{TEM-1b}, bla_{CMY-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*, *sitABCD*, *vat*	FOX, CHL, TET, AXO, AUG2, XNL, FIS, SXT, AMP, STR	*strA*, *strB*, *aadA2*, bla_{TEM-1b}, bla_{CMY-2}, *floR*, *sul1*, *sul2*, *tetA*, *dfrA12*	IncAC2, IncX1
DP26-1	ST10	*H24*	O157:H16	EPEC	*celb*, *eae*, *eitABCD*, *espA*, *espB*, *espF*, *nleB*, *nleC*, *sepA*, *tccP*, *tir*	CTX, AXO, XNL, FIS, AMP	$bla_{CTX-M-24}$	IncFIB/FII
DP26-2A	ST224	*H61*	O8:H23	None	*gad*, *lpfA*	FOX, CAZ, TET, AXO, AUG2, CIP, NAL, FIS, AMP	*aac(6′)Ib-cr*, bla_{OXA-1}, bla_{CMY-2}, bla_{TEM-1b}, *catB3*, *tetB*	IncFIA/FII/FIB

(*cnf1*, *vat*, *cvaAB*, *fyuA*, *irp2*, *ibeA*, *papC*, *iroBCDEN* and *sitABCD*); *cnf1* is a defining trait of necrotoxigenic *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 atypical 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 cefotaxime-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.

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