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## Multidrug-resistant *Escherichia coli* from canine urinary tract infections tend to have commensal phylotypes, lower prevalence of virulence determinants and *ampC*-replicons<sup>☆</sup>

Samuel Wagner, David L. Gally, Sally A. Argyle<sup>\*</sup>

Royal Dick School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Roslin, Midlothian EH25 9RG, United Kingdom

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## ABSTRACT

Multidrug-resistant *Escherichia coli* is an emerging clinical challenge in domestic species. Treatment options in many cases are limited. This study characterized MDR *E. coli* isolates from urinary tract infections in dogs, collected between 2002 and 2011. Isolates were evaluated in terms of  $\beta$ -lactamase production, phylogenetic group, ST type, replicon type and virulence marker profile. Comparisons were made with antibiotic susceptible isolates also collected from dogs with urinary tract infections. AmpC  $\beta$ -lactamase was produced in 67% of the MDR isolates (12/18). Of these, 8 could be specifically attributed to the CMY-2 gene. None of the isolates tested in either group expressed ESBLs. Phylo-group distribution was as expected in the susceptible isolates, with an over representation of the pathogenic B2 phylo-group (67%). In contrast, the phylogenetic background for the MDR group was mixed, with representation of commensal phylo-groups A and B1. The B2 phylo-group represented the smallest proportion (A, B1, B2 or D was 28%, 22%, 11% and 33%, respectively). Virulence marker profiles, evaluated using Identibac<sup>®</sup> microarray, discriminated between the two groups. Marker sequences for a core panel of virulence determinants were identified in most of the susceptible isolates, but not in most of the MDR isolates. These findings indicate that for MDR isolates, plasmid-mediated AmpC is an important resistance mechanism, and while still capable of causing clinical disease, there is evidence for a shift towards phylogenetic groups of reduced inferred virulence potential. There was no evidence of zoonotic potential in either the susceptible or MDR urinary tract isolates in this study.

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### 1. Introduction

Increasing numbers of reports have documented the emergence of *Escherichia coli* capable of producing broad-spectrum  $\beta$ -lactamases. This is significant since the  $\beta$ -lactam antimicrobials are of therapeutic importance in

humans and many domestic animals. Furthermore, many isolates are resistant to additional antimicrobial classes and are therefore multidrug-resistant (MDR).

Carriage of ESBL- and AmpC-producing *E. coli* has been documented in many species (Bortolaia et al., 2011). Antimicrobial use has been reported as a risk factor (Damborg et al., 2011, 2012; Maddox et al., 2012) and there is also evidence of sharing of organisms between species living in close proximity to each other (Dolejska et al., 2011). Considering the physical closeness in which many humans live with their pet companions, sharing of these organisms between humans and pets could pose a significant mutual risk.

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<sup>\*</sup> Corresponding author. Tel.: +44 131 6513910.

E-mail address: [sally.argyle@ed.ac.uk](mailto:sally.argyle@ed.ac.uk) (S.A. Argyle).

Clinical disease associated with these organisms is well documented in humans. Initially the pattern was of hospital-acquired infection but community-acquired infection has become increasingly important. ESBL *E. coli* are associated with a variety of clinical diseases, in particular urinary tract infections, neonatal septicaemia and wound infections (Ben-Ami et al., 2009; Pitout, 2010). Reports of similar clinical disease associated with AmpC-producing *E. coli* are far fewer than those pertaining to ESBLs, however these also appear to be an emerging problem (Oteo et al., 2010).

Although most animal studies have focused on the zoonotic risk posed by carriage, there are increasing reports demonstrating the involvement of these organisms in clinical disease in domestic species in a variety of locations including Europe, North America, Asia and Australia. For example, in the United States AmpC (CMY-2) and ESBL-producing *E. coli* (O'Keefe et al., 2010; Sanchez et al., 2002; Shaheen et al., 2011) have been reported from canine clinical isolates. Clinical disease associated with AmpC-producing *E. coli* in dogs in Australia, was first reported in 2006 (Sidjabat et al., 2006). More recently, a survey of clinical isolates from dogs and horses in the Netherlands demonstrated a 2% prevalence of ESBL and AmpC-producing isolates (Dierikx et al., 2012).

The aim of this study was to evaluate the association of ESBL or AmpC production with multidrug-resistant *E. coli* isolated from clinical cases of urinary tract infection in dogs from a local patient population, over a period of time ranging from 2002 to 2011. Isolates were further characterized in terms of phylogenetic grouping, sequence type and virulence genotype. Plasmid replicon typing was also performed to identify the type and diversity of plasmids involved. Comparisons were made to a group of susceptible *E. coli* isolates, also associated with canine urinary tract infection and collected over a similar time frame.

## 2. Materials and methods

### 2.1. Source of clinical isolates

All 15 susceptible and 17/18 MDR isolates were identified in clinical cases seen at the Hospital for Small Animals, University of Edinburgh. One MDR isolate (R3) came from a local practice serviced by the University of Edinburgh's diagnostic microbiology service.

### 2.2. *E. coli* identification

A total of 33 clinical isolates from canine urinary tract infections were cultured on Blood and MacConkey agar. Any lactose fermenting colonies were confirmed as *E. coli* utilizing biochemical testing (API 10S<sup>®</sup> strip bioMérieux).

### 2.3. Antimicrobial susceptibility testing

Susceptibility testing was performed using the disc diffusion method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines. The following discs were used: Co-trimoxazole (25 µg); ciprofloxacin (1 µg);

amoxicillin clavulanate (30 µg); cephalexin (30 µg); gentamicin (10 µg); tetracycline (10 µg); cefotaxime (30 µg). All discs were sourced from Mast group Ltd.

### 2.4. Control strains

The following control strains were utilized in both the phenotypic combination disc testing and for polymerase chain reaction. ATCC 25922<sup>™</sup> (negative control); ATCC BA-199<sup>™</sup> (SHV-3 positive control); NCTC 13353<sup>™</sup> (CTX-M-15 positive control), and NCTC 13351<sup>™</sup> (TEM-3 positive control).

### 2.5. Combination disc method for plasmid-mediated AmpC and ESBL detection

A commercially available AmpC and ESBL detection set (Mast group Ltd) was utilized. This comprised a set of 4 discs containing cefpodoxime plus or minus AmpC and ESBL inhibitors. Interpretation was made following the manufacturer's instructions.

### 2.6. PCR for ESBL and AmpC gene detection

DNA from single colonies of each isolate was prepared using the lysis method as previously described (Pérez-Pérez and Hanson, 2002). Primers for the genes *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX</sub>, *bla*<sub>CMY-1</sub> group, *bla*<sub>CMY-2</sub> group, *bla*<sub>OXA-1</sub> group and *bla*<sub>OXA-2</sub> group were derived from a previously established assay (Hasman et al., 2005). For additional detection of AmpC β-lactamase genes, multiplex PCR was performed on all samples using the methodology and primers previously described (Pérez-Pérez and Hanson, 2002). PCR products were electrophoresed in a 1% agarose gel, gel bands were excised, and sequences were compared to the NCBI database to confirm identity.

### 2.7. Multiplex PCR phylogenetic grouping of clinical isolates

DNA from single colonies was prepared using the Qiagen DNeasy blood and tissue extraction kit according to the manufacturer's instructions (Qiagen, UK). Multiplex PCR methodology was employed to assign the clinical isolates to one of four phylogenetic groups (A, B1, B2 or D). Primers and methodology have been described previously (Doumith et al., 2012).

### 2.8. PCR-based plasmid replicon typing of clinical isolates

DNA was isolated as described above for the phylogenetic grouping. Methodology involved the use of 8 multiplex reactions in a commercial kit (Diatheva, Italy) based on methodology described previously (Carattoli et al., 2005).

### 2.9. Identibac<sup>®</sup> microarray analysis

A microarray assay developed and carried out by the Animal Health Veterinary Laboratories Agency (AHVLA) was used (Batchelor et al., 2008). The microarray contained a selection of oligonucleotide probes mapping to a range of

resistance and virulence-associated genes. Probe hybridizations resulting in signal intensities greater than 0.4 were considered positive indicating the presence of the gene.

### 2.10. MLST methodology

DNA was extracted using a DNeasy extraction kit (Qiagen) and performed as to the manufacturer's instructions. Sequencing of the DNA was carried out on an Illumina MiSeq (ARK Genomics). Raw sequence reads were aligned to two reference sequences, *E. coli* ABU83972 and *E. coli* MG1655 (Accession numbers NC\_017631.1 and NC\_000913.2), using BWA and Samtools (Li and Durbin, 2009). Sequence type calling (for multi-locus sequence typing) was performed using SRST (Inouye et al., 2012). Isolates which could not be typed using SRST were called manually using sequences mapped to MG1655. Sequences aligning to the MLST genes in MG1655 were extracted using VCFtools (Danecek et al., 2011) and Extractseq (Rice, 2000) and entered manually into the MLST Database, hosted by University College Cork (<http://mlst.ucc.ie/mlst/dbs/Ecoli>).

### 2.11. Statistical methods

Comparisons were tested using Fisher's exact test. The criterion for statistical significance was taken to be  $P < 0.05$ .

## 3. Results

### 3.1. Culture and sensitivity

Between 2002 and 2011, 18 *E. coli* isolates associated with urinary tract infections (UTIs) in 16 dogs were identified as multidrug-resistant (MDR). Results of culture

and sensitivity testing for the MDR isolates (R1–16) are displayed in Table 1. Two of the dogs had recurrent UTI infection. One case recurred 1 month later (R16a and b) and the second case 6 months later (R11a and b). The criterion used to make the MDR determination was resistance to 3 or more classes of antimicrobial on routine culture and sensitivity testing. All MDR isolates were resistant to amoxicillin clavulanate and tetracycline, 83% (15/18) were resistant to cephalixin, 78% (14/18) were resistant to co-trimoxazole, 56% (10/18) were resistant to ciprofloxacin, 22% (4/18) were resistant to gentamicin and 67% (12/18) were resistant to the 3rd generation cephalosporin cefotaxime. The latter was used as an indicator of broad-spectrum  $\beta$ -lactamase production.

For comparison, 15 *E. coli* isolates were selected for study, based on the criteria that they were susceptible to all 7 antimicrobial classes listed above. These isolates were associated with urinary tract infections in dogs.

### 3.2. AmpC $\beta$ -lactamase phenotype and genotype

To identify the contribution of either ESBL or AmpC  $\beta$ -lactamases to the MDR phenotype, all isolates (both MDR and susceptible groups) were tested using the 4 disc test as described in materials and methods. All susceptible isolates were negative (data not shown). A total of 67% (12/18) of MDR isolates were positive for AmpC production. ESBL production was not detected.

Using *ampC* multiplex PCR, 9/12 isolates phenotypically AmpC positive were genotypically positive for pAmpC (CITM group). Further simplex PCR identified 8 of these to be specifically associated with the CMY-2 gene. Identibac<sup>®</sup> microarray also detected a CMY gene signal in 8/9 isolates tested (isolates relate to R1–R7, R9 and R10 in Table 1).

**Table 1**  
Summary of phenotypic and genotypic characterization of the MDR *E. coli* isolates.

Isolate	Isolation date (month/year)	Resistance pattern					3rd Gen	AmpC Pheno	pAmpC PCR	Phylo	ST	Replicon type	
R1	9/2006	AMC	CEF	COT	CIP	TET	R	+	+	A	10	FII I1	
R2	1/2008	AMC	CEF	COT		TET	R	+	+	n/t	46	FII I1	
R3	9/2010	AMC	CEF	COT	CIP	TET	R	+	+	A	744	FII I1	
R4	10/2010	AMC	CEF			TET	R	+	+	D	648	FII I1	
R5	3/2010	AMC	CEF			TET	R	+	+	D	963	FII I1	
R6	2/2011	AMC	CEF	COT		TET	R	+	+	D	N	FII I1	
R7	12/2007	AMC	CEF	COT	CIP	TET	R	+	+	B1	539	B/O I1	
R8	9/2011	AMC	CEF	COT	CIP	TET	GEN	S	–	B1	23	FII FIB	
R9	9/2011	AMC	CEF	COT		TET	R	+	+	B1	101	I1	
R10	3/2002	AMC	CEF	COT	CIP	TET	R	+	+	B2	167	FII FIA I1	
R11a	2/2010	AMC	CEF	COT		TET	R	+	–	D	10	–	
R11b	8/2010	AMC	CEF			TET	R	+	–	D	372	–	
R12	7/2006	AMC	CEF	COT		TET	GEN	S	n/d	D	372	I2	
R13	8/2009	AMC		COT	CIP	TET	S	–	n/d	A	10	FII FIA	
R14	8/2008	AMC		COT	CIP	TET	S	–	n/d	A	10	–	
R15	4/2009	AMC	CEF	COT	CIP	TET	S	–	n/d	A	998	–	
R16a	4/2011	AMC	CEF		CIP	TET	GEN	R	–	n/d	B1	23	FII B/O
R16b	5/2011	AMC		COT	CIP	TET	GEN	S	–	n/d	B2	23	FII FIB

Resistance pattern identifies antimicrobials to which organisms were resistant: AMC = Amoxicillin clavulanate; CEF = Cephalixin; COT = co-trimoxazole; CIP = ciprofloxacin; TET = tetracycline; GEN = gentamicin. 3rd Gen identifies which organisms were resistant (R) or sensitive (S) to the 3rd generation cephalosporin cefotaxime. AmpC Pheno identifies which isolates were positive or negative for AmpC on the 4 disc phenotypic test. AmpC PCR identifies those isolates where plasmid associated AmpC genes could be identified by PCR (n/d designates not done), Phylo represents phylogenetic group, ST represents sequence type and N represents a novel ST type.

### 3.3. Phylogenetic group and ST designation

PCR-based phylogenetic grouping was performed to assign the isolates to one of 4 phylogenetic groups, namely A, B1, B2 or D. Phylotypes A and B1 are considered to be associated with commensal status or intestinal pathotypes, while B2 and D are more commonly associated with strains causing extraintestinal infections (Tenailon et al., 2010). Among the susceptible isolates B2 was the predominant phylogenetic group (10 isolates, 67%) with no isolates in group A, 2 isolates (13%) in the B1 grouping and 3 isolates (20%) in group D. In contrast, the MDR group showed a more even distribution among all 4 phylogenetic groups (1 isolate could not be typed), with B2 comprising the smallest category. The distribution for A, B1, B2 or D was 28%, 22%, 11% and 33% respectively. The proportion of MDR isolates identified as phylotype B2 differed significantly from the proportion of susceptible isolates identified as B2 ( $P < 0.001$ ).

MLST identified 18 ST types among the *E. coli* isolates. Within the MDR group these were: ST10 ( $n = 4$ ); ST 23 ( $n = 3$ ); ST372 ( $n = 2$ ); ST46 ( $n = 1$ ); ST744 ( $n = 1$ ); ST648 ( $n = 1$ ); ST963 ( $n = 1$ ); ST539 ( $n = 1$ ); ST101 ( $n = 1$ ); ST167 ( $n = 1$ ); and ST 998 ( $n = 1$ ). The two MDR isolates belonging to phylogenetic group B2 belonged to ST types 167 and 23. The overall association of ST type and phylogenetic group is listed in Table 1. Within the susceptible group ST types identified were: ST73 ( $n = 4$ ); ST12 ( $n = 2$ ); ST641 ( $n = 1$ ); ST 127 ( $n = 1$ ); ST10 ( $n = 1$ ); ST625 ( $n = 1$ ); ST929 ( $n = 1$ ); and ST3005 ( $n = 1$ ). A total of 3 isolates from the susceptible group and 1 from the MDR group did not map to existing ST types. None of the isolates belonged to ST131.

### 3.4. Identibac<sup>®</sup> microarray analysis

A panel of 11 specific probes was extracted from a much larger panel. These represent probes for which any isolate, either susceptible or MDR, demonstrated a positive result. The full list of probes against which isolates were tested can be found in the supplementary materials.

The virulence marker panel results are summarized in Table 3. Although the number of isolates examined was limited, there were significantly higher ( $P < 0.05$ ) levels of carriage demonstrated for 7/11 specific virulence markers in the susceptible group compared to the MDR group.

### 3.5. Plasmid replicon typing

In the susceptible group (Table 2) plasmid replicons could not be identified in 5 of the isolates. In the remaining 10 isolates, 6 isolates had 2 or more replicons and 4 isolates carried single replicons. The FII replicon was present in 5 isolates and the FIB replicon was present in 4 isolates. Overall 8 different replicon types were identified in this group. In the MDR group (Table 1), plasmid replicons could not be identified in 4 of the isolates. A total of 12 of the remaining 14 carried 2 or more replicons and only 2 isolates carried single replicons. The FII replicon was present in 11 isolates and the I1 replicon was present in 9 isolates. A total of 6 isolates carried the FII and I1 replicons together (these 6 isolates were all phenotypically and

**Table 2**

Summary of phylogenetic group and replicon type of the susceptible urinary tract isolates.

Isolate	Isolation date (month/year)	Phylo	ST	Replicon type
S1	1/2003	B2	73	–
S2	10/2011	B2	73	–
S3	11/2001	B2	12	–
S4	11/2001	B2	73	–
S5	11/2001	B2	N	–
S6	1/2001	B1	641	X1
S7	10/2002	B2	12	I2 R
S8	1/2003	D	N	FIA
S9	1/2003	B2	127	FIB
S10	12/2011	B2	10	FII
S11	12/2000	B2	73	FII FIB
S12	10/2011	D	N	FII FIB
S13	1/2002	D	625	FII FIB B/O
S14	8/2001	B2	929	FII I1
S15	9/2001	B1	3005	B/O I1

Phylo represents phylogenetic group, ST represents sequence type and N represents novel sequence types.

genotypically positive for pAmpC). Overall 6 different replicon types were identified in this group.

## 4. Discussion

These findings demonstrate clinically significant MDR *E. coli* in canine urinary tract infections. The antibiogram phenotype of isolates (Table 1.) shows that treatment options are limited. All MDR isolates were resistant to the recommended first line treatment amoxicillin clavulanate and more than half of the isolates were resistant to fluoroquinolones, a third line option (Weese et al., 2011).

MDR strains were analyzed in some detail. Comparisons were made to a susceptible group of canine UTI isolates from the same locality and indirectly to significant human clonal lineages. The latter is particularly important in light of concerns regarding the transfer of organisms between humans and domestic animals, and the potential for either to act as a reservoir of infection for the other.

AmpC rather than ESBL-producing *E. coli* were commonly identified among the MDR isolates. This is interesting because, in human UTIs associated with MDR *E. coli*, ESBLs (particularly the CTX-M group) seem to be the predominant enzymes responsible for broad-spectrum resistance to  $\beta$ -lactams. Although this study has a low number of isolates, other studies have also identified the presence of AmpC producing *E. coli* in dogs associated with both faecal carriage and clinical disease (Damborg et al., 2011; Dierikx et al., 2012; Murphy et al., 2009; Shaheen et al., 2011; Sidjabat et al., 2006; Tamang et al., 2012; Wedley et al., 2011). Furthermore, routine screening in our laboratory has continued to identify AmpC-producing isolates causing urinary tract infection (10 isolates from January 2012 to November 2013). Although this represents a relatively low local incidence, these cases are still clinically significant and therapeutically challenging.

The phylogenetic profile differed between the two groups of isolates. The susceptible group of UTI isolates predominantly belonged to the B2 phylogenetic group, as predicted from previous studies in humans and dogs



**Table 3**  
Identification of virulence markers in the MDR and susceptible *E. coli* isolates. X indicates presence of the gene.

Isolate	Phyl	ireA	iroN	iss	mchB	mchC	mchF	mcm	perA	prfB	senB	sfaS
			a	a	a	a	a	a		a		
R1	A											
R2	n/t											
R3	A		X	X								
R4	D			X								
R5	D											
R6	D											
R7	B1											
R8	B1	X	X	X			X	X		X		
R9	B1		X	X	X	X	X					
R10	B2											
R11a	D											
R11b	D											
R12	D				X							
R13	A							X		X		
R14	A											
R15	A											
R16a	B1											
R16b	B2		X	X			X	X		X		
S1	B2	X	X	X	X	X	X	X				
S2	B2		X	X	X	X	X	X		X	X	
S3	B2	X	X	X	X	X	X	X		X		
S4	B2		X	X	X	X	X	X		X		
S5	B2	X	X	X	X	X	X	X		X		
S6	B1		X	X	X	X	X	X				
S7	B2		X	X	X	X	X			X		
S8	D		X	X	X	X				X		
S9	B2		X	X	X			X		X		X
S10	B2	X	X	X	X	X	X	X	X	X	X	X
S11	B2											
S12	D		X	X				X		X		
S13	D		X		X	X	X				X	
S14	B2	X	X	X	X	X	X	X		X		X
S15	B1											

R = MDR isolate; S = susceptible isolate; Phyl = phylogroup; ireA = siderophore receptor; iroN = enterobactin siderophore receptor; iss = increased serum survival; mchB = microcin H47 part of colicin H; mchC = MchC protein; mchF = ABC transporter protein; mcm = microcin M part of colicin H; perA = EPEC adherence factor; prfB = P-related fimbriae regulatory gene; senB = plasmid encoded enterotoxin; sfaS = S fimbriae minor subunit.

<sup>a</sup> Identifies significant difference for MDR versus susceptible.

(Johnson et al., 2003; Mao et al., 2012; Thompson et al., 2011). The MDR group meanwhile demonstrated a more even distribution across all four phylo-groups, with significantly less representation of the B2 phylo-group. It is worth noting that a more recent methodology has been able to assign *E. coli* to 8 rather than 4 phylo-groups (Clermont et al., 2013), which could have altered the profile of the isolates in this study, and future work should employ the revised methodology to provide greater detail and depth of characterization. In the context of the results from this study, isolates already assigned to B1 or B2 would be unlikely to change classification, so the difference between groups in the proportions of isolates in phylotype B2 should still be valid.

The virulence marker profile also differed between the two groups, suggesting a reduced virulence genotype in the MDR isolates compared to the susceptible ones. Previous studies have indicated that, in MDR isolates associated with UTI in both humans and dogs, there may be a shift away from the dominance of the B2 phylogenetic group and a decrease in certain virulence genes (Moreno et al., 2006; Vila et al., 2002). The reason for this pattern is unclear. Other researchers have speculated that less

pathogenic phylogenetic groups are more receptive to the acquisition of the MDR phenotype (Johnson et al., 2004), or that acquisition of the MDR phenotype results in a trade off, with a loss of virulence traits. Whatever the order of events, it would seem logical that less pathogenic phylogenetic groups, with an MDR phenotype, would require certain conditions under which to cause clinical disease.

Sequence typing did not provide evidence for clonal spread of isolates in either group. Considering the extended sampling time this is probably not surprising. More pertinent perhaps was the fact that ST131 was not identified amongst any of our isolates. The O25b-ST131 clonal lineage is one of the most important uropathogenic *E. coli* groups in humans. It belongs to the B2 phylogenetic group, is multidrug-resistant (almost always resistant to the fluoroquinolones and often resistant to 3rd generation cephalosporins), is often, but not always, an ESBL producer (CTXM-15) and is highly virulent (Oteo et al., 2010; Thompson et al., 2011). Total reports of ST131 in domestic animals are still extremely low and, although there is some support for interspecies transfer of ST131, it is unclear if animals are a major

**Table 4**Clinical summary of the cases from which MDR *E. coli* were isolated.

Isolate	Breed	Age (years)	Summary of clinical details
R1	Dachshund	10	Surgery to treat intervertebral disc prolapse. Amoxicillin/clavulanate used for surgical prophylaxis.
R2	Weimeraner	9	Prostatic abscess. Surgical drainage and antibiotics (enrofloxacin and clindamycin).
R4	Cocker Spaniel	4	Immune mediated haemolytic anaemia, which was treated with immunosuppressant drugs.
R5	Cross breed	11	Hyperadrenocorticism. Amoxicillin/clavulanate administered for several months to manage concurrent liver disease.
R6	Boxer	9	Multiple mast cell tumours. Resection followed by cancer chemotherapy.
R7	Schnauzer	6	Mast cell tumour. Surgical management followed by cancer chemotherapy.
R8	Labrador	10	Surgery to resect intestinal adenocarcinoma. Post surgery developed pneumonia and treated with multiple antibiotics.
R9	Collie	10	Diabetes mellitus poorly controlled.
R10	German Shepherd	8	Prostatic infection and a perineal hernia accompanied by recurrent cystitis.
R11	Bouvier de Flandres	6	Placement of a gastrostomy tube. The stoma became infected requiring antimicrobials. Subsequently the dog developed recurrent urinary tract infections.
R12	Bearded Collie	1	Pyelonephritis. Initially treated with amoxicillin/clavulanate.
R13	Cocker Spaniel	7	Pancreatitis
R14	Golden Retriever	5	Ectopic ureters possibly acting as a predisposing factor for urinary tract infection.
R15	Yorkshire Terrier	11	Concurrent urolithiasis.
R16	Boxer cross	7	Detrusor muscle atony decreasing the ability to empty the bladder during urination.

reservoir or incidental host of this ExPEC clonal lineage, or indeed if humans act as an animal reservoir (Platell et al., 2011). Certainly within the limitations of our study we found no evidence for this.

One MDR isolate (R4) was typed as ST648 phylogenetic group D. Strains of this clonal lineage that carry ESBLs, have been associated with bacteraemias in human patients in the Netherlands and NDM carbapenamases in human patients in the United Kingdom and Pakistan (Tamang et al., 2012).

Plasmid replicon typing was performed in order to establish the range and diversity of plasmids amongst both the MDR and susceptible isolates. Most of the plasmid replicons correspond to incompatibility groups. In the susceptible group replicon types could not be assigned to 5 of the isolates. This is perhaps not surprising, as the assay is designed to detect resistance plasmids. In the MDR group there were 4 isolates that were not assigned replicon types. Interestingly 2 of these (R11a and b) were phenotypically AmpC-producing, but it was not possible to detect plasmid-associated genes. We speculate that AmpC production in these isolates could be attributed to chromosomal mutations in the *ampC* promoter. Further analysis of these isolates will be required to confirm this. In the remainder of the MDR isolates the FII and I1 replicon types were the most highly represented. We observed a cluster comprising 6 isolates (R1–R6 Table 1) all AmpC-producing and carrying Inc FII/IncI1 plasmids. Since IncFII and IncI1 plasmids are two of a number of plasmid types that are particularly successful in their ability to spread multidrug resistance (Carattoli, 2011), further characterization and comparison of the plasmids from these isolates would be of particular interest.

With the exception of one case for which we have no history, all cases caused by MDR *E. coli* had underlying disease involving suppression of the immune system (e.g. hyperadrenocorticism, cancer chemotherapy), an anatomical abnormality of the genitourinary tract (e.g. detrusor muscle atony, ectopic ureters), and/or a history of prior antimicrobial treatment. Clinical details are summarized

in Table 4. This is not a surprising finding since it can be envisaged that such factors will increase the potential of isolates, which we speculate may be less virulent, to cause clinical disease. What will be of interest is to follow the natural history of infections caused by these organisms in companion animals. It is highly probable that future changes in the epidemiology of MDR *E. coli* infections in dogs, will reflect those seen in the human population, where there has been a shift from hospital-acquired infection, analogous to what we have observed in this study in dogs, to a community-acquired infection, where they are associated with uncomplicated urinary tract disease.

The main limitations of this study are the low number of MDR isolates, and the relatively extended time over which samples were collected. However, this reflects the relative scarcity of such MDR isolates associated with routine community-acquired canine urinary tract infections tested over the last decade at our laboratory, with the majority of the MDR isolates arising from more complex clinical cases at the small animal hospital.

## 5. Conclusion

MDR *E. coli* are a cause of a small but clinically significant number of urinary tract infections in dogs serviced by the veterinary microbiology service at Edinburgh University.

Broad-spectrum  $\beta$ -lactamase production is an important resistance mechanism and, unlike in human infections, AmpC rather than ESBL production predominates. This may reflect the gut carriage of AmpC *E. coli* in the dog. Amongst our isolates MDR *E. coli* are skewed towards less pathogenic phylogenetic groups and have a reduced virulence genotype. The variety of ST types does not support clonal spread; horizontal transfer of certain plasmid types may be a more important mechanism for the transmission of the MDR phenotype, judging by the frequency of the IncFII and IncI1 plasmid types. Most importantly, ST type analysis of both susceptible and MDR

isolates suggests that dogs with urinary tract infection are not acting as a reservoir for zoonotic spread. Neither does it provide evidence for reverse zoonosis from the human population.

### Conflict of interest

The authors have no financial or personal relationships with organizations or people that could inappropriately influence or bias their work

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetmic.2014.01.003>.

### References

- Batchelor, M., Hopkins, K.L., Liebana, E., Slickers, P., Ehrlich, R., Mafura, M., Aarestrup, F., Mevius, D., Clifton-Hadley, F.A., Woodward, M.J., Davies, R.H., Threlfall, E.J., Anjum, M.F., 2008. Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. *Int. J. Antimicrob. Agents* 31, 440–451.
- Ben-Ami, R., Rodríguez-Baño, J., Arslan, H., Pitout, J.D.D., Quentin, C., Calbo, E.S., Azap, O.K., Arpin, C., Pascual, A., Livermore, D.M., Garau, J., Carmeli, Y., 2009. A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. *Clin. Infect. Dis.* 49, 682–690.
- Bortolaia, V., Larsen, J., Damborg, P., Guardabassi, L., 2011. Potential pathogenicity and host range of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* isolates from healthy poultry. *Appl. Environ. Microb.* 77, 5830–5833.
- Carattoli, A., 2011. Plasmids in Gram negatives: molecular typing of resistance plasmids. *Int. J. Med. Microbiol.* 301, 654–658.
- Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., Threlfall, E.J., 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63, 219–228.
- Clermont, O., Christenson, J.K., Denamur, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Rep.* 5, 58–65.
- Damborg, P., Gaustad, I.B., Olsen, J.E., Guardabassi, L., 2011. Selection of CMY-2 producing *Escherichia coli* in the faecal flora of dogs treated with cephalixin. *Vet. Microbiol.* 151, 404–408.
- Damborg, P., Marskar, P., Baptiste, K.E., Guardabassi, L., 2012. Faecal shedding of CTX-M-producing *Escherichia coli* in horses receiving broad-spectrum antimicrobial prophylaxis after hospital admission. *Vet. Microbiol.* 154, 298–304.
- Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. *Bioinformatics* 27, 2156–2158.
- Dierikx, C.M., van Duijkeren, E., Schoormans, A.H.W., van Essen-Zandbergen, A., Veldman, K., Kant, A., Huijsdens, X.W., van der Zwaluw, K., Wagenaar, J.A., Mevius, D.J., 2012. Occurrence and characteristics of extended-spectrum- $\beta$ -lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *J. Antimicrob. Chemother.* 67, 1–7.
- Dolejska, M., Duskova, E., Rybarikova, J., Janoszowska, D., Roubalova, E., Dibdakova, K., Maceckova, G., Kohoutova, L., Literak, I., Smola, J., Cizek, A., 2011. Plasmids carrying blaCTX-M-1 and qnr genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre. *J. Antimicrob. Chemother.* 66, 757–764.
- Doumith, M., Day, M.J., Hope, R., Wain, J., Woodford, N., 2012. Improved multiplex PCR strategy for rapid assignment of the four major *Escherichia coli* phylogenetic groups. *J. Clin. Microbiol.* 50, 3108–3110.
- Hasman, H., Mevius, D., Veldman, K., Olesen, I., Aarestrup, F.M., 2005. Beta-lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in the Netherlands. *J. Antimicrob. Chemother.* 56, 115–121.
- Inouye, M., Conway, T.C., Zobel, J., Holt, K.E., 2012. Short read sequence typing (SRST): multi-locus sequence types from short reads. *BMC Genomics* 13, 338.
- Johnson, J.R., Kaster, N., Kuskowski, M.A., Ling, G.V., 2003. Identification of urovirulence traits in *Escherichia coli* by comparison of urinary and rectal *E. coli* isolates from dogs with urinary tract infection. *J. Clin. Microbiol.* 41, 337–345.
- Johnson, J.R., Kuskowski, M.A., Gajewski, A., Sahm, D.F., Karlowsky, J.A., 2004. Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of *Escherichia coli* from across the United States, 2000–2001. *J. Infect. Dis.* 190, 1739–1744.
- Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25, 1754–1760.
- Maddox, T.W., Pinchbeck, G.L., Clegg, P.D., Wedley, A.L., Dawson, S., Williams, N.J., 2012. Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 2: Risk factors for faecal carriage of antimicrobial-resistant *Escherichia coli* in horses. *Equine Vet. J.* 44, 297–303.
- Mao, B.-H., Chang, Y.-F., Scaria, J., Chang, C.-C., Chou, L.-W., Tien, N., Wu, J.-J., Tseng, C.-C., Wang, M.-C., Chang, C.-C., Hsu, Y.-M., Teng, C.-H., 2012. Identification of *Escherichia coli* genes associated with urinary tract infections. *J. Clin. Microbiol.* 50, 449–456.
- Moreno, E., Prats, G., Sabaté, M., Pérez, T., Johnson, J.R., Andreu, A., 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. *J. Antimicrob. Chemother.* 57, 204–211.
- Murphy, C., Reid-Smith, R.J., Prescott, J.F., Bonnett, B.N., Poppe, C., Boerlin, P., Weese, J.S., Jancko, N., McEwen, S.A., 2009. Occurrence of antimicrobial resistant bacteria in healthy dogs and cats presented to private veterinary hospitals in southern Ontario: a preliminary study. *Can. Vet. J.* 50, 1047–1053.
- O’Keefe, A., Hutton, T.A., Schifferli, D.M., Rankin, S.C., 2010. First detection of CTX-M and SHV extended-spectrum beta-lactamases in *Escherichia coli* urinary tract isolates from dogs and cats in the United States. *Antimicrob. Agents Chemother.* 54, 3489–3492.
- Oteo, J., Cercenado, E., Cuevas, O., Bautista, V., Delgado-Iribarren, A., Orden, B., Pérez-Vázquez, M., García-Cobos, S., Campos, J., 2010. AmpC beta-lactamases in *Escherichia coli*: emergence of CMY-2-producing virulent phylogroup D isolates belonging mainly to STs 57, 115, 354, 393, and 420, and phylogroup B2 isolates belonging to the international clone O25b-ST131. *Diagn. Microbiol. Infect. Dis.* 67, 270–276.
- Pérez-Pérez, F.J., Hanson, N.D., 2002. Detection of plasmid-mediated AmpC<sub>NL</sub>-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* 40, 2153–2162.
- Pitout, J.D.D., 2010. Infections with extended-spectrum beta-lactamase-producing enterobacteriaceae: changing epidemiology and drug treatment choices. *Drugs* 70, 313–333.
- Platell, J.L., Johnson, J.R., Cobbold, R.N., Trott, D.J., 2011. Multi-drug resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST 131 in animals and foods. *Vet. Microbiol.* 153, 99–108.
- Rice, P., 2000. The European molecular biology open software suite EMBOSS: the European molecular biology open software suite 9525, 2–3.
- Sanchez, S., McCrackin Stevenson, M.A., Hudson, C.R., Maier, M., Buffington, T., Dam, Q., Maurer, J.J., 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J. Clin. Microbiol.* 40, 3586–3595.
- Shaheen, B.W., Nayak, R., Foley, S.L., Kweon, O., Deck, J., Park, M., Rafii, F., Boothe, D.M., 2011. Molecular characterization of resistance to extended-spectrum cephalosporins in clinical *Escherichia coli* isolates from companion animals in the United States. *Antimicrob. Agents Chemother.* 55, 5666–5675.



- Sidjabat, H.E., Townsend, K.M., Hanson, N.D., Bell, J.M., Stokes, H.W., Gobius, K.S., Moss, S.M., Trott, D.J., 2006. Identification of bla (CMY-7) and associated plasmid-mediated resistance genes in multi-drug-resistant *Escherichia coli* isolated from dogs at a veterinary teaching hospital in Australia. *J. Antimicrob. Chemother.* 57, 840–848.
- Tamang, M.D., Nam, H.-M., Jang, G.-C., Kim, S.-R., Chae, M.H., Jung, S.-C., Byun, J.-W., Park, Y.H., Lim, S.-K., 2012. Molecular characterization of extended-spectrum- $\beta$ -lactamase-producing and plasmid-mediated AmpC  $\beta$ -lactamase-producing *Escherichia coli* isolated from stray dogs in South Korea. *Antimicrob. Agents Chemother.* 56, 2705–2712.
- Tenaillon, O., Skurnik, D., Picard, B., Denamur, E., 2010. The population genetics of commensal *Escherichia coli*. *Nat. Rev. Microbiol.* 8, 207–217.
- Thompson, M.F., Litster, A.L., Platell, J.L., Trott, D.J., 2011. Canine bacterial urinary tract infections: new developments in old pathogens. *Vet. J.* 190, 22–27.
- Vila, J., Simon, K., Ruiz, J., Horcajada, J.P., Velasco, M., Barranco, M., Moreno, A., Mensa, J., 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? *J. Infect. Dis.* 186, 1039–1042.
- Wedley, A.L., Maddox, T.W., Westgarth, C., Coyne, K.P., Pinchbeck, G.L., Williams, N.J., Dawson, S., 2011. Prevalence of antimicrobial-resistant *Escherichia coli* in dogs in a cross-sectional, community-based study. *Vet. Rec.* 168, 354.
- Weese, J.S., Blondeau, J.M., Boothe, D., Breitschwerdt, E.B., Guardabassi, L., Hillier, A., Lloyd, D.H., Papich, M.G., Rankin, S.C., Turnidge, J.D., Sykes, J.E., 2011. Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. *Vet. Med. Int.* 2011, 263768.