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Citation for published version:

Wedley, AL, Dawson, S, Maddox, TW, Coyne, KP, Pinchbeck, G, Clegg, PD, Nuttall, T, Kirchner, M & Williams, N 2016, 'Carriage of antimicrobial resistant *Escherichia coli* in dogs: prevalence, associated risk factors and molecular characteristics', *Veterinary Microbiology*, vol. 199, pp. 23-30.
<https://doi.org/10.1016/j.vetmic.2016.11.017>

Digital Object Identifier (DOI):

[10.1016/j.vetmic.2016.11.017](https://doi.org/10.1016/j.vetmic.2016.11.017)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Veterinary Microbiology

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Accepted Manuscript

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PII: S0378-1135(16)30666-6
DOI: <http://dx.doi.org/doi:10.1016/j.vetmic.2016.11.017>
Reference: VETMIC 7445

To appear in: *VETMIC*

Received date: 8-9-2015
Revised date: 6-11-2016
Accepted date: 16-11-2016

Please cite this article as: Wedley, Amy L., Dawson, Susan, Maddox, Thomas W., Coyne, Karen P., Pinchbeck, Gina L., Clegg, Peter, Nuttall, Tim, Kirchner, Miranda, Williams, Nicola J., Carriage of antimicrobial resistant *Escherichia coli* in dogs: Prevalence, associated risk factors and molecular characteristics. *Veterinary Microbiology* <http://dx.doi.org/10.1016/j.vetmic.2016.11.017>

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Carriage of antimicrobial resistant *Escherichia coli* in dogs: prevalence, associated risk factors and molecular characteristics.

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Research Highlights

- Antimicrobial resistant (AMR) *E. coli* from faeces of vet-visiting dogs were common.
- Risk factors for carriage include antimicrobial use and consuming raw poultry meat.
- Resistance genes identified are also common in isolates of human origin.
- Dogs may be a reservoir of AMR bacteria, having public health implications.

Abstract

Resistance to antimicrobials, in particular that mediated by extended spectrum β -lactamases (ESBL) and AmpC β -lactamases are frequently reported in bacteria causing canine disease as well as in commensal bacteria, which could be a potential health risk for humans they come into contact with. This cross-sectional study aimed to estimate the prevalence and investigate the molecular characteristics of ESBL and plasmid encoded AmpC (pAmpC)-producing *E. coli* in the mainland UK vet-visiting canine population and, using responses from detailed questionnaires identify factors associated with their carriage. Faecal samples were cultured for antimicrobial resistant (AMR), ESBL and pAmpC-producing *E. coli*. A subset of ESBL and pAmpC-producing isolates were subjected to multi-locus sequence typing and DNA microarray analyses. Multivariable logistic regression analysis was used to construct models to identify risk factors associated with multidrug resistant (MDR, resistance to three or more antimicrobial classes), fluoroquinolone resistance, ESBL and AmpC-producing *E. coli*. AMR *E. coli* were isolated from 44.8% (n=260) of samples, with 1.9% and 7.1% of samples carrying ESBL and pAmpC-producing *E. coli*, respectively. MDR *E. coli* were identified in 18.3% of samples. Recent use of antimicrobials and being fed raw poultry were both identified as risk factors in the outcomes investigated. A number of virulence and resistance genes were identified, including genes associated with extra-intestinal and enteropathogenic *E. coli* genotypes. Considering the close contact that people have with dogs, the high levels of AMR *E. coli* in canine faeces may be a potential reservoir of AMR bacteria or resistance determinants.

1. Introduction

Antimicrobial resistant (AMR) bacteria is a common and increasing problem in healthcare and community settings. Multidrug resistant bacteria, for example *Escherichia coli*, are commonly reported in dogs and other companion animals (Shaheen et al., 2010). In addition

to *E. coli* being a common cause of gastrointestinal infections, the vast majority of humans, dogs and other mammals carry commensal *E. coli* within the gut. However, such commensal bacteria may also cause opportunistic disease if outside their normal niche, for example in the urinary tract (Johnson and Russo, 2002). Furthermore, the location of commensal *E. coli* means that they are exposed to the selective pressure of antimicrobials given orally. These commensal bacteria may then themselves act as a reservoir for such resistance determinants. As such, *E. coli* isolated from faecal samples can provide a good indication of the reservoir of resistance within the gut flora (van den Bogaard and Stobberingh, 2000).

One resistance mechanism of particular concern is that mediated by extended spectrum β -lactamases (ESBLs), which hydrolyse third generation cephalosporins, such as ceftazidime, cefotaxime, cefpodoxime and ceftiofur (Livermore, 2008). *E. coli* harbouring such ESBL encoding genes have become increasingly prevalent in hospitals and in the community in people (Brolund et al., 2014) and dogs (Gandolfi-Decristophoris et al., 2013). Plasmid mediated AmpC (pAmpC) enzymes, which also have a broad spectrum of resistance to cephalosporins, but are resistant to β -lactamase inhibitors, have also been documented in dogs (Sidjabat et al., 2006).

While AMR *E. coli* from canine clinical samples has been reported (Pedersen et al., 2007), the general human population is more likely to be exposed to the bacteria present in the faeces of dogs. For example, owners picking up after their dogs have defecated. It is therefore important to understand the prevalence of antimicrobial resistance among the *E. coli* resident in the gut of healthy dogs. Such studies have been carried out in both Europe (SVARM, 2006) and Canada (Murphy et al., 2009) and have demonstrated that animals and humans often carry the same resistance determinants. A small number of studies have shown, using macro-restriction pulsed-field gel electrophoresis, that dogs, cats and humans in the same household can have identical bacteria (Johnson et al., 2008a), supporting the suggestion of

transmission of enteric bacteria between humans and their pets. However, studies that investigate the risk factors for carriage of AMR bacteria are very limited and focus mainly on acquisition of antimicrobial resistance during hospitalisation (Gibson et al., 2011; Leite-Martins et al., 2014). Identification of such risk factors could be key in developing effective strategies to reduce the development and spread of such resistance in the future.

The aim of this study was to determine the faecal prevalence of AMR *E. coli*, including ESBL and pAmpC β -lactamase producing *E. coli*, in the mainland UK vet-visiting dog community. DNA micro array analysis was used on a subset of multi drug resistant (MDR, resistant to three or more antimicrobial classes), and ESBL producing isolates to investigate the presence of genes associated with antimicrobial resistance and virulence. In addition, risk factors associated with carriage of antimicrobial resistance were determined.

2. Materials and Methods

2.1. Study population

Dogs visiting veterinary practices in mainland UK were recruited during consultation with their clinician. Practices were randomly selected from those listed in the 2006 RCVS directory of veterinary practices who indicated that they treated dogs. Hospitalised dogs were excluded. An estimated sample size of 555 dogs was based on the participation of 50 practices, an expected carriage rate of AMR *E. coli* of 50%, 5% precision, 95% confidence intervals and a conservative between practice variance of 0.01. To allow for a degree of non-compliance, each practice was asked to recruit 28 dogs. A further 37 practices were subsequently recruited due to the low numbers of dogs enrolled by some practices. Dogs were recruited to the study between April 2008 and July 2009. Consent was obtained, from the owners, by the clinician during their routine consultation before any samples were collected. Ethical approval was granted by the University of Liverpool Committee on Research Ethics (Reference RETH000118).

2.2. Sample collection and questionnaire

Owners were provided with a sterile collection tube and gloves to collect a fresh faecal sample from their dog. They were also asked to complete a six-page questionnaire with both tick box and free text questions. The questionnaire included questions relating to signalment, medical history of the dog over the previous three months (including use of antimicrobials), diet and recent use of antimicrobials by any other family member (including other pets). The faecal sample and completed questionnaire were returned by first class post.

2.3. Sample processing

An equal volume of the faecal sample was added to 5 ml of brain heart infusion broth with 5% glycerol (BHIG) and mixed thoroughly to create a faecal homogenate. A portion of this was stored below -70 °C and the remainder used to isolate AMR *E. coli*, as previously described (Wedley et al., 2011). Briefly, the faecal homogenate was plated directly onto MacConkey and eosin methylene blue agar (EMBA) and antimicrobial discs (MAST group Ltd) applied to the surface: ampicillin (10 µg); amoxicillin clavulanic acid (30 µg); chloramphenicol (30 µg); ciprofloxacin (1 µg); nalidixic acid (30 µg); tetracycline (30 µg); and trimethoprim (2.5 µg). Following overnight incubation at 37°C, colonies morphologically consistent with *E. coli* within the zone of inhibition around the antimicrobial discs were selected. One isolate, if present, from within the zone of each of the seven antimicrobial discs was selected. For screening of samples for ESBL producing *E. coli*, two EMBA plates, one containing cefotaxime (1 µg/ml) and the other ceftazidime (1 µg/ml), were streaked with the faecal homogenate. If present, at least one isolate morphologically consistent with *E. coli* was selected from each plate. If no growth consistent with *E. coli* occurred, further EMBA plates were streaked with faecal homogenate following overnight enrichment in buffered peptone water. In addition, for non-selective isolation of *E. coli*, an EMBA plate containing no antimicrobials was streaked with the faecal homogenate. Three

isolates morphologically consistent with *E. coli* were selected for antimicrobial susceptibility testing. Therefore, it was possible to select a total of 19 isolates from each sample. The identity of *E. coli* isolates was confirmed by biochemical testing and a PCR assay to detect the *uidA* gene (McDaniels et al., 1996).

2.4. Antimicrobial susceptibility testing

Antimicrobial disc susceptibility testing following British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Andrews, 2007) was performed on all isolates using the same antimicrobial discs as used for the isolation of *E. coli* above. Additionally, for potential ESBL or AmpC producing *E. coli*, an extended panel of antimicrobial discs were used: aztreonam (30µg), ceftazidime (30µg), ceftriaxone (30µg), ceftiofur (30µg), cefuroxime (30µg), cefalexin (30µg), tazobactam (10µg), piperacillin (75µg) and trimethoprim-sulfamethoxazole (25µg). The reference strain *E. coli* ATCC 25922 was used as a fully sensitive control in all testing. Following overnight incubation at 37°C, the zone diameters in mm were recorded. For each sample, only one isolate per unique resistance phenotype was included in any subsequent testing.

2.5. Phenotypic confirmation of ESBL production

The paired disc diffusion test (MAST Group Ltd) was performed on isolates suspected of ESBL production, selected from the EMBA plates containing ceftazidime or cefotaxime. Following overnight incubation, the zone diameters in mm were recorded. Production of an ESBL by an isolate was confirmed if the zone diameter was expanded by at least 5mm in the presence of clavulanic acid. When an isolate showed resistance to a cephalosporin, with a difference in zone diameter less than 5mm this suggested the production of an AmpC β -lactamase, both an ESBL and an AmpC β -lactamase, or an inhibitor resistant ESBL.

2.6. Characterisation of ESBL and *bla*_{AmpC} genes

PCR was used to detect the presence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and plasmid encoded *bla*_{AmpC} genes as previously described (Batchelor et al., 2005; Boyd et al., 2004; Essack et al., 2001; Perez-Perez and Hanson, 2002). For *bla*_{CTX-M} positive isolates, assignment of the specific *bla*_{CTX-M} gene group was carried out (Batchelor et al., 2005; Boyd et al., 2004), followed by sequencing of the PCR amplicon. For *bla*_{CMY} positive isolates, the gene was amplified (Liebana et al., 2004), with an additional set of internal primers (CITMf and CITMr) used for sequencing (Perez-Perez and Hanson, 2002). All sequences were compared to those submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) to determine the specific *bla*_{CTX-M} or *bla*_{CMY} gene. In all PCRs, a positive control, known to harbour the target gene in previous PCR assays, was included.

2.7. DNA microarray analysis of *E. coli*

Isolates for DNA microarray analysis were chosen due to their phenotypic resistance to multiple classes of antimicrobials and included all isolates positive for carriage of a *bla*_{CTX-M} gene and a selection of those carrying *bla*_{TEM} and/or plasmid encoded *bla*_{AmpC} genes. These *E. coli* isolates were subjected to DNA microarray based chip analysis using Alere *E. coli* and Alere AMR-ve chips according to the manufacturer's instructions (Alere Technologies, Jena, Germany). Alere *E. coli* array detects the presence of virulence associated *E. coli* genes. Alere AMR-ve detects the presence of genes responsible for resistance to a selection of antimicrobials in Gram negative bacteria.

2.8. Multi-Locus Sequence Typing analysis of *E. coli*

Multi-locus sequence typing (MLST) of the isolates selected for DNA microarray analysis was carried out as previously described (Wirth et al., 2006), <http://mlst.ucc.ie/mlst/dbs/Ecoli>.

2.9. Statistical analysis

Four different outcomes (fluoroquinolone resistance, MDR, ESBL-producing *E. coli* and pAmpC-producing *E. coli*) were tested for associations with explanatory variables

(questionnaire responses) using univariable logistic regression. In all models, clustering at the practice level was taken into account by including the practice as a mixed effect in the models constructed. A likelihood ratio test (LRT) comparing the models with and without each of the explanatory variables was performed. Associations were considered statistically significant if $P < 0.05$. For variables with small numbers, categories were grouped together. Also, the reference category was changed if this was considered appropriate. For example, for length of prescription of antimicrobials, “none prescribed” was made the reference category. For variables with zero cells, a Fisher’s exact test was carried out to determine if there was a significant association between the outcome and the response variable. If significant, a false positive was added to the dataset for the purposes of construction of a multivariable model. A multivariable model was constructed including all those variables with a P-value less than 0.3. Backwards elimination was used where removal of each variable from the full model as assessed by the likelihood ratio test and the variable with the highest P-value was removed. This process was repeated until only those variables whose removal resulted in a LRT P-value of less than 0.05 remained. Each variable that had been removed was then sequentially added back in and a LRT performed to test whether the variable should remain in the final model. Where the final model included more than two variables, tests for interactions between the variables were carried out and interactions with P-value of less than 0.05 were retained in the final model. Multivariable analysis excluded any dogs that did not have complete responses for any of the variables included in the model. Statistical analyses were performed using the R base, lme4, and lmerTest packages.

To determine the intraclass correlation coefficient (the measure of clustering of an outcome within practices), the latent variable approach was used (Goldstein et al., 2002) with equation 1 below.

$$\text{ICC} = 100 \times \frac{\text{Practice variance}}{\text{Practice variance} + \frac{\pi^2}{3}} \quad (\text{Equation 1})$$

3. Results

3.1. Study population

In total, 580 faecal samples, 574 with questionnaires, were returned but only 445 questionnaires were completed fully. The median age of dogs recruited was 5 years (range 6 weeks to 17 years) and 64 different pure breeds were represented. Cross-breeds (n=123, 21.4%), followed by Labrador retrievers (n=90, 15.7%) were most common.

3.2. Prevalence of antimicrobial resistance

At least one *E. coli* was isolated from 561 (96.7%) of the 580 faecal samples, with AMR *E. coli* isolated from 260 (44.8%) faecal samples. A total of 436 unique *E. coli* isolates (up to nine per sample) were recovered based on their antimicrobial susceptibility profile. Table 1 shows the simple sample prevalence of faecal carriage of AMR *E. coli* and the prevalence adjusted for clustering. The most common resistance phenotypes observed were to ampicillin (37.2%), tetracycline (30.0%) and trimethoprim (23.8%). *E. coli* resistant to amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin and nalidixic acid was observed in less than 10% of dogs. MDR *E. coli* was isolated from 18.3% of dogs. The intraclass correlation coefficient (ICC) of presence of ESBL and pAmpC genes (53.5% and 19.9% respectively) among *E. coli* is much higher than for resistance to the other antimicrobials (between 0 and 10%), suggesting some degree of clustering at the practice level for these outcomes.

ESBL producing *E. coli* were isolated from 1.9% of samples and a pAmpC β -lactamase producing *E. coli* from 7.1% of samples. Fifty-two isolates from fifty dogs (8.6% of samples) were identified as either ESBL or pAmpC producers by phenotypic testing and PCR. In these isolates, resistance to ampicillin, cephalexin and ceftiofur (8 isolates) was most common. All

but four isolate were susceptible to aztreonam. MDR was observed in 13 of the 52 isolates (25%).

3.3. Characterisation of ESBL and AmpC β -lactamase genes

Of the 58 isolates obtained from the cephalosporin containing plates, 21 carried a *bla*_{TEM} gene. However, six of these were not resistant to third generation cephalosporins in disc diffusion testing and are therefore most likely to be *bla*_{TEM-1}. Ten carried a *bla*_{CTX-M} gene (Table 2), most of which belonged to CTX-M group 1 (five *bla*_{CTX-M-1}, two *bla*_{CTX-M-15} and one *bla*_{CTX-M-3}). One isolate carried *bla*_{CTX-M-14/18} (CTX-M group 9) and one *bla*_{CTX-M-20} (CTX-M group 2). A plasmid encoded *bla*_{AmpC} gene was found in 42 isolates, which were all shown to be *bla*_{CMY-2}. No isolates carried a *bla*_{SHV} gene.

3.4. Microarray analysis of *E. coli*

A subset of 30 isolates underwent microarray analysis. These included all ten isolates harbouring a *bla*_{CTX-M} gene and a selection of isolates harbouring one or both of *bla*_{TEM} or *bla*_{AmpC}. The results observed with AMR array chip showed good agreement with *in vitro* testing of antimicrobial susceptibility (Figure 1). Of the 30 isolates tested, 23 harboured genes encoding resistance to tetracycline (*tet(A)* and *tet(B)*) and 20 harboured genes encoding resistance to trimethoprim (*dfrA1*, *dfrA7*, *dfrI2*, *dfrA17* and *dfrA5*). Genes encoding resistance to aminoglycosides were also identified in 25 isolates (*aadA1*, *aadA2*, *aadA4*, *aac3Iva*, *aac6Ib*, *strA* and *strB*), whilst 24 isolates harboured genes encoding resistance to sulphonamides (*sul1* and *sul2*). Seven isolates harboured genes encoding resistance to chloramphenicol (*catA1*, *catB3* and *floR*). Erythromycin resistance genes (*ere(A)* and *erm(B)*) were identified in a single isolate. A number of resistance genes appeared to be clustered within isolates. For example, there was a positive correlation between trimethoprim resistance genes *dfrA19* and *dfrA17* and the aminoglycosidase gene *aadA4*. Correlation was also evident between the aminoglycosidase genes *strA* and *strB* and the sulphonamide

resistance gene *sul2*. Supplementary figure F1 provides further detail of the clustering present between resistance genes in the isolates investigated.

Twenty-three different genes associated with virulence were detected. Ten or isolates were found to be positive for *iss* (22 isolates) associated with increased resistance to serum, followed by *iroN* (14 isolates) associated with iron uptake, *lpfA* (11 isolates) encoding long polar fimbriae and *mchF* (10 isolates) encoding a subunit of an ABC transporter protein. The full list of virulence genes identified is provided in supplementary table S1. A number of genes associated with the locus of enterocyte effacement (LEE) were detected in eight isolates, though none appeared to possess all genes encoded by the pathogenicity island.

3.5. Multi-Locus Sequence Typing

The 31 isolates studied by microarray also underwent multi-locus sequence typing, although seven could not be typed due to poor sequence quality, or because no amplicon could be obtained. In total, 19 different sequence types (STs) were identified (Figure 1). Sixteen occurred only once and three (ST1684, ST1710 and ST1832) were novel sequence types. The most commonly identified STs were ST963 (three isolates), ST88 (two isolates) and ST1670 (two isolates). With the exception of the three ST963 isolates, which all carried *bla*_{CMY} gene, no association with ST and specific *bla*_{CTX-M} or *bla*_{CMY} genes was evident. The two isolates that carried *bla*_{CTX-M-15} were found to be ST410 and ST448.

3.6. Risk factors associated with carriage of AMR *E. coli*

Univariable analysis identified a variety of explanatory variables to be significantly associated with one or more of the four outcomes tested. Any explanatory variables with a p-value of less than 0.3 were included in the initial multivariable model (Supplementary tables 1 to 4). With the exception of being fed raw poultry, which was significantly associated with ESBL carriage (P<0.001), fluoroquinolone resistance (P<0.001) and MDR (P=0.006), no variables were associated with more than one of the outcomes.

Following sequential removal of variables from the maximal model, Table 3 shows the variables remaining in the models of the four outcomes. Raw poultry consumption remained significantly associated with ESBL carriage (OR 48.04), fluoroquinolone resistance (OR 2.18) and MDR (OR 4.11). There was evidence of an interaction between raw poultry consumption and being a working dog in the fluoroquinolone resistance model with working dogs that also consumed raw poultry being 104 times more likely to carry an *E. coli* that was resistant to fluoroquinolones, however confidence intervals were wide. Breed size was identified as a risk factor for ESBL carriage and MDR. For ESBL mediated resistance with large dogs eight times more likely to carry ESBL producing *E. coli* compared to small/medium dogs. In the MDR model, large dogs were 2.5 times more likely to carry MDR *E. coli* than medium sized dogs with no significant difference between small and medium dogs. Other variables that remained in the final models included antimicrobial prescription, presence of or contact with other animals, age and the purpose of the veterinary consultation, though none were associated with more than one of the four models.

4. Discussion

This study investigated the prevalence faecal carriage of AMR *E. coli* by vet visiting dogs in the UK. Plasmid mediated AmpC β -lactamase-producing *E. coli* carriage was higher (7.1%) than the prevalence of ESBL producing *E. coli* (1.9%). However, only one gene variant associated with the AmpC β -lactamase phenotype (*bla*_{CMY-2}) was identified, which has been identified in *E. coli* from canine clinical samples in Italy and Canada (Carattoli et al., 2005; Sanchez et al., 2002), and healthy dogs from many countries including the USA and the UK (Murphy et al., 2009; Wedley et al., 2011). The high frequency of this gene in canine isolates may be due to spread of a few specific plasmids (Hopkins et al., 2006), or the integration of the *bla*_{CMY-2} gene into many plasmids facilitating widespread dissemination (Carattoli et al., 2002). There was a greater clustering of pAmpC carriage (19.9%, the degree of clustering)

within practice compared to other outcomes (with the exception of ESBL carriage, 53.53%), which supports the suggestion that prescribing practices (Hughes et al., 2012), or other practice specific factors, may have a direct effect of carriage of AMR bacteria.

This study found carriage of AMR *E. coli* to be common among the vet-visiting dogs (44.8%) with resistance to ampicillin, tetracycline and trimethoprim most prevalent, reflecting previous studies (Costa et al., 2008; SVARM, 2006), which may be attributed to the numerous determinants responsible for resistance and that they are readily transmissible, but also the frequent use of these and related drugs in dogs .

Resistance to other antimicrobials in this study appeared to be higher than previously reported (Carattoli et al., 2005; Costa et al., 2008; Murphy et al., 2009; Wedley et al., 2011). This may be due to the type of population; for example these were vet-visiting dogs, some of which reported recent antimicrobial use, while other studies recruited community dogs and/or excluded animals with any history of antimicrobial use. MDR isolates were found in 18.4% of dogs; however, it is difficult to compare this with other studies since the definition of MDR differs between studies.

Microarray analysis of antimicrobial resistance genes showed that many isolates harboured genes encoding resistance to a wide range of antimicrobials, including sulphonamides (*sul2*), aminoglycosides (*aadA1*, *strA*) and chloramphenicol (*catA1*). These AMR genes are also commonly identified in isolates of human origin (Card et al., 2015; Frye et al., 2006; Kirchner et al., 2014). Furthermore, the predominant genes and mechanisms of resistance for each of the antimicrobials concur with other studies of resistance genes in canine isolates (Costa et al., 2008; Lanz et al., 2003). This provides further evidence that antimicrobial resistance in bacteria isolated from dogs is often mediated by the same genes as those of human origin. Whilst microarray analysis was only carried out on a subset of the isolates, there was strong evidence co-carriage of different resistance genes. Plasmids are known to

carry numerous resistance genes and so the presence of correlation of gene carriage is to not unexpected.

A wide variety of genes associated with virulence were also detected, but by far the most commonly identified were those associated with extra-intestinal pathogenic *E. coli* (ExPEC) including *iss* and *iroN* (Johnson et al., 2008c; Russo et al., 2002). However, the microarray chip did not include the specific genes that have been suggested as being markers of ExPEC (at least two of the five virulence markers: *papA* and *papC*, *sfa/foc*, *afa/dra*, *iutA*, and *kpsMT II*), (Johnson et al., 2001). Dogs have been suggested as a potential reservoir for the ExPEC pathotype (Johnson et al., 2008b). It is also interesting to note that some isolates harboured virulence genes associated with the locus of enterocyte effacement (LEE) found in enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) (Kaper et al., 2004). Dogs often eat faeces of livestock, which are carriers of EHEC, furthermore a previous study found coprophagy to be a risk factor for antimicrobial resistance in canine faecal samples (Leite-Martins et al., 2014).

Many of the STs identified have previously been identified in both clinical and non-clinical samples from humans, dogs and other animals from within the UK, other parts of Europe and as far as Canada and Australia (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/GetTableInfo.html>). For example, ST10 has been reported in the UK, Africa, Europe, South America and the USA in samples from humans, dogs and livestock. Recently, a new human clone has been reported (Schaufler et al., 2016), which has also been identified in veterinary clinical isolates in the UK (Timofte et al., 2016), with an isolate of the same ST (ST410) resistant to fluoroquinolones and carrying *bla*_{CTX-M-15} found in this study. Typing of more isolates with a diverse range of resistance phenotypes may enable better links between ST, virulence and resistance to be investigated further. There was a high degree of diversity among ESBL and AmpC β -lactamase-producing isolates in this study, demonstrating that such *E. coli* show

highly variable antimicrobial resistance, virulence and sequence types, but overall was similar to that observed in human isolates.

This study determined some risk factors associated with carriage of AMR *E. coli* in the UK dog population. The finding that administration of antimicrobials within the last 3 months was associated with AMR *E. coli* carriage in two of the final models is not surprising given the selection pressure this would exert upon commensal bacteria. Previous studies found that the administration of fluoroquinolones and β -lactams were associated with an increased risk of carriage of AMR *E. coli* in dogs (Gibson et al., 2011; Leite-Martins et al., 2014). Further work in this area may provide greater understanding of the effects of short and long term antimicrobial use on the gut flora, selection and carriage of resistant bacteria in dogs.

Eating raw poultry was also identified as a risk factor in the final model of two of the three outcomes tested. This was also identified in a study investigating AMR *E. coli* in faecal samples from Labrador retrievers (Schmidt et al., 2015). Chickens have been identified as a potential reservoir for resistant bacteria and determinants (Costa et al., 2009) and it is possible that ingestion of raw poultry results in transfer of these. This is also supported by five of the ten isolates positive for *bla*_{CTX-M} carrying *bla*_{CTX-M-1}, which has been identified as the most common gene variant present in isolates of poultry origin (Efsa, 2011). Furthermore, a study in the Netherlands observed a high prevalence of ESBL carriage in raw food (Overdeest et al., 2011), demonstrating an increased risk of carriage of AMR bacteria when dogs consume raw meat.

Limitations in this study may include the method of recruitment and selection bias, for example, during busy periods owners may not have been approached and case selection was based on a non-random convenience population. This cross sectional study gives the prevalence of carriage of AMR *E. coli* at a single time point, and it may be possible that this is transient in nature. A study in the Netherlands (Baede et al., 2015), repeatedly sampled 38

dogs over six months and found that many of the dogs demonstrated a high degree of variation between faecal shedding of AmpC and ESBL producing *E. coli*. Repeated sampling may represent a more accurate method to determine faecal shedding by dogs. Breed size also remained significantly associated with ESBL producing and MDR *E. coli*. However, the breed size of 161 dogs was not specified either because the owners did not specify the breed of their dog, or the dog was a cross breed and the size could not be determined.

In conclusion, many of the dogs sampled harboured AMR *E. coli*. The potential for dogs to act as reservoirs for AMR bacteria and determinants has implications for public health. The welfare of the individual dogs could also be affected if this restricts future treatment options. In addition, knowledge of potential risk factors associated with the carriage of antimicrobial *E. coli* by dogs in the community will allow better risk management. Educating owners on the risks of raw food diets comprising poultry meat and regulation of the process in addition to ensuring the prudent use of antimicrobial by veterinary surgeons would be a good place to begin.

5. Acknowledgments

The research was funded by the Department for Environment, Food and Rural Affairs (Defra) project OZ0612. The authors thank Ruth Ryvar and Gill Hutchinson for technical assistance. The authors would like to thank the veterinary practices and their staff for recruitment of owners. Finally, we wish to convey our gratitude to the owners of the dogs for agreeing to their dogs' participation in the study.

6. Conflict of interest statement

None

7. References

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8. Figure Legends

Figure 1. Heat-map analysis the presence of antimicrobial resistance genes among ESBL and AmpC β -lactamase producing *E. coli* from canine faecal samples by DNA micro array. Black indicates that the isolate was positive for the gene.

A - Quinolone resistance, B - Sulphonamides, C - Tetracyclines, D - Aminoglycosides, E - Chloramphenicol, F - Trimethoprim, G - β -lactams, H - Erythromycin, I - Streptogramin, ST – Multi-locus sequence type.

Supplementary figure F1. Correlation matrix of antimicrobial resistance genes identified in *E. coli* isolated from vet visiting canine faecal samples.

A - Quinolone resistance, B - Sulphonamides, C - Tetracyclines, D - Aminoglycosides, E - Chloramphenicol, F - Trimethoprim, G - β -lactams, H - Erythromycin, I - Streptogramin, ST – Multi-locus sequence type.

Table 1. Prevalence of antimicrobial resistant *E. coli* isolated from 581 canine faecal samples.

	Sample prevalence % (95% confidence interval) n=580	Adjusted prevalence* % (95% confidence interval)	ICC
Any resistance	44.8% (40.8-48.9)	44.7% (40.1-49.4)	3.07
Multidrug resistance	18.3% (15.1-21.4)	17.6% (14.1-21.4)	5.02
Ampicillin	37.2% (33.3-41.2)	36.9% (32.2-41.9)	5.57
Amoxicillin/clavulanic acid	7.1% (5.0-9.2)	6.6% (4.3-10.0)	5.21
Chloramphenicol	9.1% (6.8-11.5)	8.0% (5.5-11.6)	10.62
Ciprofloxacin	5.0% (3.2-6.8)	5% (3.5-7.1)	0
Nalidixic acid	7.9% (5.7-10.1)	7.9% (6.0-10.4)	0
Tetracycline	30% (26.3-33.7)	28.9% (24.3-33.9)	7.54
Trimethoprim	23.8% (20.3-27.3)	23.2 (19.5-27.5)	4.03
ESBL mediated resistance	1.9% (0.8-3.0)	0.5% (0.1-3.3)	53.53
AmpC mediated resistance	7.1% (5.0-9.2)	5.1% (3.0-8.8)	19.93

*Estimates adjusted for clustering within practice

Table 2. Genes detected and AMR phenotype in 52 ESBL or AmpC producing *E.coli* isolates from canine faecal samples.

Genes detected	Row Labels	Count of Full profile
<i>bla</i> _{CMY2}	Amp, 1st Gen	1
<i>bla</i> _{CMY2}	Amp, 1st Gen, 2nd Gen	1
<i>bla</i> _{CMY2}	Amp, Amc, 1st Gen	2
<i>bla</i> _{CMY2}	Amp, Amc, 1st Gen, 2nd Gen	1
		7
<i>bla</i> _{CMY2}	Amp, Amc, 1st Gen, 2nd Gen, 3rd Gen	3
<i>bla</i> _{CMY2}	Amp, Amc, Tet, 1st Gen, 2nd Gen	3
<i>bla</i> _{CMY2}	Amp, Atm, Sxt, 1st Gen, 2nd Gen, 3rd Gen	1
<i>bla</i> _{CTX-M-1}	Amp, Tet, 1st Gen, 2nd Gen, 3rd Gen	1
<i>bla</i> _{CTX-M-1}	Amp, Tmp, Sxt, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, 1st Gen, 2nd Gen	3
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, 1st Gen, 2nd Gen, 3rd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Atm, Cip, Nal, Tmp, Sxt, Tet, Chl, 1st Gen, 2nd Gen, 3rd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Atm, Tmp, Sxt, Tet, 1st Gen, 2nd Gen, 3rd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Cip, Nal, Tmp, Sxt, Tet, 1st Gen, 2nd Gen	2
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Cip, Nal, Tmp, Sxt, Tet, Chl, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Cip, Nal, Tmp, Tet, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Tet, 1st Gen, 2nd Gen	2
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Amc, Tmp, Sxt, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CMY2}	Amp, Tmp, Sxt, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1}	Amp, Nal, Tmp, Sxt, Tet, Chl, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1}	Amp, Tmp, Sxt, Tet, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1}	Amp, Tmp, Sxt, Tet, Chl, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-14/18}	Amp, Tmp, Sxt, Tet, Chl, 1st Gen, 2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-15}	Amp, Atm, Cip, Nal, Tmp, Sxt, Tet, 1st Gen2nd Gen3rd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-15}	Amp, Cip, Nal, Tmp, Sxt, Tet, 1st Gen2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-3}	Amp, Tmp, Sxt, Tet, 1st Gen2nd Gen	1
<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-20}	Amp, Tmp, Sxt, Tet, 1st Gen2nd Gen	1

*1st Gen cephalixin, 2nd Gen cefuroxime/ cefoxitin, 3rd Gen ceftazidime/ ceftriaxone, Chl chloramphenicol, Mon aztreonam, Pen ampicillin/ amoxicillin/clavulanic acid, Q ciprofloxacin/ nalidixic acid, Tet tetracycline, Tm trimethoprim/ co-trimoxazole.

Table 3. Multivariable model of variables associated with carriage of an ESBL (TEM or CTX-M), AmpC, Multi-drug resistant or fluoroquinolone resistant *E. coli*. Practice (n=73) was included as a random effect.

		AmpC mediated resistance (565 observations)		ESBL mediated resistance (560 observations)		MDR (571 observations)		Fluoroquinolone resistance (538 observations)	
Variable	Response	OR (95% CI)	P-Value	OR (95% CI)	P-Value	OR (95% CI)	P-Value	OR (95% CI)	P-Value
Prescribed any antibiotic in previous three months	No	Ref	<0.001						
	Yes	5.99 (2.92-13.11)							
Other animals in the household	No	Ref	0.039						
	Yes	0.46 (0.20-0.96)							
Fed raw poultry	No			Ref	<0.001	Ref	0.004	Ref	<0.001
	Yes			48.04 (8.83-334.29)		4.11 (1.59-10.39)		2.18 (0.32-9.26)	
Neutered	No			Ref	0.033				
	Yes			8.76 (1.17-201.74)					
Breed size	Small			Ref (Small & Medium)	0.016	1.16 (0.48-2.70)	0.03		
	Medium					Ref			
	Large			8.32 (1.34-162.71)		2.48 (1.36-4.76)			
	Not specified			0.93 (0.03-25.95)		1.04 (0.51-2.12)			
Visited for Vaccination or worming	No			Ref	0.033				
	Yes			0.37 (0.006-0.87)					
In contact with other dogs during walks	No			Ref	0.047				
	Yes			0.21 (0.045-0.98)					
Visited with specific complaint	No					Ref	0.020		
	Yes					1.73 (1.09-2.79)			
Working dog	No							Ref	0.044
	Yes							1.28 (0.29-4.03)	
Age (years)	<1							6.40 (1.06-	0.01

		AmpC mediated resistance (565 observations)	ESBL mediated resistance (560 observations)	MDR (571 observations)	Fluoroquinolone resistance (538 observations)
Variable	Response	OR (95% CI) P-Value	OR (95% CI) P-Value	OR (95% CI) P-Value	OR (95% CI) P-Value
	1 to 2 3 or more				55.5 Ref 6.46 (1.55-47.7)
Sex	Male Female				Ref 0.016 2.39 (1.17-5.13)
Medication prescribed during most recent visit	No Yes				Ref 0.036 2.08 (1.05-4.16)
Fed titbits	Never/ rarely Sometimes/ often				Ref 0.014 2.17 (1.05-4.99)
Raw poultry: Working dog*	No raw poultry, not a working dog Fed raw poultry and is a working dog				Ref 0.005 37.49 (2.74-866.10)
Practice Variance (standard deviation) ICC		0.755 (0.869) 18.66%	0 (0)	0.17(0.416) 4.99%	0(0)
Deviance		221.41	66.67	491.47	252.04
Degrees of freedom of model residuals		561	552	564	528
P-value (Chi-squared)		1.000	1.00	0.987	1

***Explanation of interaction term.** No raw poultry not a working dog Odds ratio = 1.No raw poultry working dog Odds ratio = 1.28. Fed raw poultry not a working dog odds ratio = 2.18, Fed raw poultry and working dog Odds ratio = 1.28*2.18*37.49 = 104.61.

