

Papers

Prevalence of antimicrobial-resistant *Escherichia coli* in dogs in a cross-sectional, community-based study

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The prevalence of carriage of antimicrobial-resistant (AMR) and extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* was determined in 183 healthy dogs from a semi-rural community in Cheshire. Isolates were tested against a panel of antimicrobials and by PCR to detect resistance genes. In the suspected ESBL-producing isolates, the presence of bla_{SHV} , bla_{TEM} , bla_{CTX-M} and bla_{AmpC} genes was determined by PCR and sequencing. A total of 53 (29 per cent, 95 per cent confidence interval [CI] 22.4 to 35.5 per cent) dogs carried at least one AMR *E coli* isolate. Twenty-four per cent (95 per cent CI 17.9 to 30.2 per cent) of dogs carried isolates resistant to ampicillin, 19.7 per cent (95 per cent CI 13.9 to 25.4 per cent) to tetracycline and 16.9 per cent (95 per cent CI 11.5 to 22.4 per cent) to trimethoprim. A bla_{TEM} gene was detected in 39 of 54 ampicillin-resistant isolates, a $tet(B)$ gene in 12 of 45 tetracycline-resistant isolates, and a dfp gene in 22 of 33 trimethoprim-resistant isolates. Multidrug-resistant isolates were demonstrated in 15 per cent (28 of 183; 95 per cent CI 10.1 to 20.5 per cent) of dogs. Nine suspected ESBL-producing *E coli* were isolated, of which only one was confirmed by double disc diffusion testing. Two of these isolates carried the bla_{TEM-1} gene and seven carried the bla_{CMY-2} gene.

ANTIMICROBIAL resistance (AMR) is a commonly encountered problem in both human and veterinary medicine. It can lead to failures in treatment, increased morbidity and mortality, and a greater financial burden on healthcare services. Use of antimicrobials may exert a selection pressure on, and therefore select for, bacteria that have acquired resistance. Such resistance can be acquired either by mutation of chromosomal DNA, or by horizontal transfer of resistance determinants via transmissible elements, such as plasmids. One particular mechanism of AMR is the production of extended-spectrum β -lactamases (ESBLs), enzymes capable of hydrolysing third-generation cephalosporins (Livermore 2008). A further important resistance

mechanism, due to their broad spectrum of resistance to β -lactams and the ineffectiveness of β -lactamase inhibitors, are plasmid-mediated AmpC enzymes (Philippon and others 2002).

Escherichia coli can be readily isolated from the gastrointestinal tract of many animal species, including human beings and dogs, and is therefore a good indicator of reservoirs of AMR (van den Bogaard and Stobberingh 2000). The presence of *E coli* in the intestinal tract of human beings, dogs and most animal species results in its exposure to any antimicrobial agents that are administered and that enter the gastrointestinal tract. This exposure can select for *E coli* that have acquired resistance determinants or mutations encoding AMR. In addition, there is the potential for AMR *E coli* to act as a reservoir of resistance determinants for pathogenic bacteria (Guardabassi and others 2004).

A significant quantity of antimicrobials sold in the UK are for veterinary use (VMD 2000). Since 2002, total veterinary sales of therapeutic antimicrobials have decreased (from 440 tonnes in 2002 to 387 tonnes in 2008); however, the total sales of drugs indicated for use in non-food-producing animals only has increased, with a notable rise in sales of therapeutic antimicrobials licensed for use in dogs only (from 4.5 tonnes in 2002 to 7.3 tonnes in 2008) (VMD 2009). Increased use of antimicrobials in dogs, coupled with selection pressures for resistance, may result in higher carriage of AMR bacteria.

People and dogs are often in close contact, and as a result there is a risk of transfer of bacteria, resistant or otherwise, from one to the other, which may influence the success of the antimicrobial treatment required by the individual if the bacteria cause disease. AMR *E coli* have been isolated from clinical samples from dogs (Normand and others 2000a, b) and other animals (Lanz and others 2003). In addition, faeces from healthy dogs (De Graef and others 2004, Costa and others 2008) and various other animals (Moyaert and others 2006) have been shown to harbour AMR *E coli*. ESBL-producing *E coli* have been isolated from both healthy dogs and those with

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TABLE 1: Primers and annealing temperatures used in PCRs for detection of antimicrobial resistance mechanisms in *Escherichia coli* isolates from faecal samples from dogs

Primer*	Sequence (5'→3')	Amplicon size (bp)	Annealing temperature (°C)	Reference
TEMF	ATGAGATTCAACATTCCCGTG	861	55	Essack and others 2001
TEMR†	TTACCAATGCTTAATCAGTGTAG			
SHVF	ATGCGTTAATATTCGCTGTG	865	60	Essack and others 2001
SHVR	GTTAGCGTTGCCAGTGTCTCG			
CTX MU1	ATGTGCAGYACAGTAARGTKATGGC	593	58	Boyd and others 2004
CTX MU2	TGGGTRAAARTARGTSACCAGAAYCAGCGG			
MOXMF‡	GCTGCTCAAGGAGCACAGGAT	520	64	Pérez-Pérez and Hanson 2002
MOXMr ¹	CACATTGACATAGGTGTGGTGC			
CITMF ^{1‡}	TGGCCAGAACTGACAGGCCAAA	462	64	Pérez-Pérez and Hanson 2002
CITMr ^{1‡}	TTTCTCCTGAACGTGGCTGGC			
DHAMF ¹	AACTTTCACAGGTGTGCTGGT	405	64	Pérez-Pérez and Hanson 2002
DHAMr ¹	CCGTACGCATACCTGGCTTTC			
ACCMF ¹	AACAGCCTCAGCAGCCGGTAA	345	64	Pérez-Pérez and Hanson 2002
ACCMr ¹	TTGCCGCAATCATCCCTAGC			
EBCMF ¹	TCGGTAAAGCCGATGTTGCGG	302	64	Pérez-Pérez and Hanson 2002
EBCMr ¹	CTTCCACTCGCGTCCGAGTT			
FOXMF ¹	AACATGGGGTATCAGGGAGATG	190	64	Pérez-Pérez and Hanson 2002
FOXMr ¹	CAAAGCGCGTAACCCGATTGG			
TetA ²	GCTACATCCTGCTTGCCTTC	210	55	Ng and others 2001
TetA ²	CATAGATCGCCGTGAAGAGG			
TetB ³	TTGGTTAGGGGCAAGTTTTTG	659	55	Ng and others 2001
TetB ³	GTAATGGGGCCAATAACACCCG			
TetC ³	CTTGAGAGCCTCAACCCAG	418	55	Ng and others 2001
TetC ³	ATGGTGTGATCTACCTGCC			
TetD ³	AAACCATTACGGCATTCTGC	787	55	Ng and others 2001
TetD ³	GACCGGATACACCATCCATC			
TetE ²	AAACCACATCTCCATACGC	278	55	Ng and others 2001
TetE ²	AAATAGGCCACAACCGTCAG			
TetG ²	GCTCGGTGGTATCTCTGCTC	468	55	Ng and others 2001
TetG ²	AGCAACAGAATCGGGAACAC			
D1 ⁴	ACGGATCCTGGCCTGTGGTGGACGC	254	55	Lee and others 2001
D2 ⁴	CGGAATTCACCTCCGGCTCGATGTC			
D3	GTTGCGGTCCAGACATAC	253	54	Lee and others 2001
D4	CCGCCACCAGACACTA			
D5	TCGAGTTCATGCCATT	454	57	Lee and others 2001
D6	TCTTCCATGCCATTCTGC			
D7 ⁵	CCGTGGGTCGATGTTGATG	485	58	Lee and others 2001
D8 ⁵	GCATTGGGAAGAAGCGCTCAC			
D9 ⁵	GTCGCCCTAAAACAAAGTTA	195	51	Lee and others 2001
D10 ⁵	CGCCCATAGAGTCAAATGT			
df ⁹ F ⁴	ATGAATCCCGTGGCATGAACAGAAAGAT	398	55	Gibreel and Sköld 1998
df ⁹ R ⁴	ATGGATCCTTCAGTAATGTCGGGACCTC			
qnrA ⁶	ATTTCTACCGCAGGATTGG	516	53	Robicsek and others 2006
qnrA ⁶	GATCGGCAAAAGGTTAGGTCA			
qnrB ⁶	GATCGTGAAAGCCAGAAAGG	469	53	Robicsek and others 2006
qnrB ⁶	ACGATGCCTGGTAGTTGTC			
qnrS ⁶	ACGACATTCGTCAACTGCAA	417	53	Robicsek and others 2006
qnrS ⁶	TAAATTTGGCACCTGTAGGC			
cmy25F	CAATGTGTGAGAAGCAGTC	1432	50	Liebana and others 2004
cmy2DR	CGCATGGGATTTCTTGCTG			
TEMIA [†]	TTCTGTGACTGTGAGTACT			Mocktar and others 2008
TEMIB [†]	GAGTAAAGTAGTTCGCCAGTT			Essack and others 2001
TEMIC [†]	CTGCAGCAATGGCAACAAC			

* Numbers next to primer name indicate primers that were used in multiplex reaction

† Primers were also used for sequencing of the *bla_{TEM}* gene

‡ Primers were also used as internal primers for sequencing of the *bla_{CMV}* gene

clinical infections (Moreno and others 2008). However, with one exception in Sweden (SVA 2006), previous studies investigating AMR *E coli* in healthy dogs have been limited in their sample size, and in some countries, including the UK, no such studies have been published.

The aim of the present study was to determine the prevalence of AMR *E coli* and ESBL-producing *E coli* in the faeces from healthy dogs in a census-based, cross-sectional study of a community in Cheshire.

Materials and methods

Collection of faecal samples

Details of the sample population and faecal sample collection from dogs in a semi-rural community in Cheshire have been previously described by Westgarth and others (2007, 2009). Briefly, in a census-based study of 1278 households, 260 were identified as dog-owning. Owners were

asked to provide a fresh faecal sample from their dog and complete a short questionnaire relating to medical history (eg, recent history of vomiting, diarrhoea and antimicrobial use). Faecal samples were obtained from 183 healthy dogs over a period from August to November 2005. The fresh faecal samples were mixed with an equal volume of brain heart infusion broth with 5 per cent glycerol and the homogenate was stored at below -70°C. The faecal homogenates were thawed and processed as described below.

Isolation and identification of *E coli*

AMR *E coli* were detected using the direct plating method previously described by Bartoloni and others (1998, 2006). Thawed faecal homogenate was plated directly on to MacConkey's agar and eosin methylene blue agar (EMBA) using a plain cotton swab, and the following antimicrobial discs (MAST Group) were applied: 10 µg ampicillin, 30 µg amoxicillin-clavulanate, 30 µg chloramphenicol, 1 µg ciprofloxacin, 30 µg nalidixic acid, 30 µg tetracycline and 2.5 µg trimethoprim. After overnight incubation at 37°C, colonies growing within the zone of inhibition around each disc on both sets of plates, whose morphology resembled *E coli* were selected for subsequent investigation. To screen for ESBL-producing *E coli*, the faecal homogenates were directly streaked on to EMBA containing 1 µg/ml ceftazidime and EMBA containing 1 µg/ml cefotaxime (Sigma-Aldrich) (Liebana and others 2006). In addition, to allow for non-selective isolation of *E coli*, faecal homogenates were also directly streaked on to EMBA containing no antimicrobials. Three isolates from this plate were selected for subsequent antimicrobial testing. Presumptive *E coli* resistant to at least one antimicrobial were confirmed by biochemical testing (Gram stain and testing for catalase production, lack of oxidase, lactose fermentation, indole production and inability to use citrate as a carbon source) and *uidA* PCR (McDaniels and others 1996). All media were supplied by Lab M.

Antimicrobial susceptibility testing

Antimicrobial disc diffusion testing was performed in accordance with British Society of Antimicrobial Chemotherapy guidelines (Andrews 2007). The following antimicrobial discs were used: 10 µg

ampicillin, 30 µg amoxicillin-clavulanate, 30 µg chloramphenicol, 1 µg ciprofloxacin, 30 µg nalidixic acid, 30 µg tetracycline and 2.5 µg trimethoprim. For the isolates suspected of ESBL production, a further panel of nine antimicrobial agents was also tested: 30 µg aztreonam, 30 µg ceftazidime, 30 µg ceftriaxone, 30 µg cefoxitin, 30 µg cefuroxime, 30 µg cefalexin, 25 µg trimethoprim-sulfamethoxazole, 10 µg gentamicin and 10 µg tazobactam/75 µg piperacillin. The reference strain *E coli* ATCC 25922 was used for quality control during testing.

Phenotypic confirmation of ESBL production

Isolates that were selected from the cephalosporin-containing EMBA plates, and therefore suspected of ESBL production, were tested using the MAST double disc diffusion method previously described by M'Zali and others (2000). Briefly, an Iso-Sensitest agar plate was inoculated with the isolate, and three pairs of cephalosporin discs (with

TABLE 2: Frequency of antimicrobial resistance in *Escherichia coli* isolates from faecal samples from 183 dogs

Antimicrobial agent	Total isolates	Number* (%)	95% CI
Ampicillin	54	44 (24.0)	17.9-30.2
Amoxicillin-clavulanate	10 (+4 int)	7 (3.8)	1.0-6.6
Chloramphenicol	5	5 (2.7)	0.4-5.1
Ciprofloxacin	4 (+2 int)	4 (2.2)	0.1-4.3
Nalidixic acid	6	6 (3.3)	0.7-5.9
Tetracycline	46	36 (19.7)	13.9-25.4
Trimethoprim	33	31 (16.9)	11.5-22.4
MDR	30	28 (15.3)	10.4-20.5

* Number of dogs carrying an isolate with resistance to at least one antimicrobial
CI Confidence interval, int Intermediate, MDR Multidrug resistance

TABLE 3: Profile of resistance among 69 *Escherichia coli* faecal isolates from 183 dogs

Resistance profile	Number (%) of isolates
AMP, TET, TMP	18 (26.1)
TET	9 (12.9)
AMP	9 (12.9)
AMP, TMP	7 (10.1)
AMP, TET	7 (10.1)
AMP, AMC, TET	4 (5.8)
TET, TMP	2 (2.9)
AMP, TMP, TET, CHL	2 (2.9)
AMP, AMC, CIP, NAL	2 (2.9)
AMP, AMC	2 (2.9)
TMP	1 (1.4)
NAL, TMP	1 (1.4)
NAL, TET	1 (1.4)
CIP, NAL, TET	1 (1.4)
AMP, TMP, CHL	1 (1.4)
AMP, AMC, CHL, TET	1 (1.4)
AMP, AMC, CHL, CIP, NAL, TET, TMP	1 (1.4)

AMC Amoxicillin-clavulanate, AMP Ampicillin, CHL Chloramphenicol, CIP Ciprofloxacin, NAL Nalidixic acid, TET Tetracycline, TMP Trimethoprim

and without clavulanic acid) were placed on the surface of the agar plate: 30 µg ceftazidime and 30 µg ceftazidime plus 10 µg clavulanic acid; 30 µg cefotaxime and 30 µg cefotaxime plus 10 µg clavulanic acid; and 30 µg cefpodoxime and 30 µg cefpodoxime plus 10 µg clavulanic acid. The plates were incubated aerobically at 37°C for 18 to 24 hours and zone diameters around each disc were measured. ESBL production was confirmed when the zone around the cephalosporin disc was expanded in the presence of the clavulanic acid by a minimum of 5 mm, according to the manufacturer's instructions (MAST Group). The AmpC phenotype was suggested when the presence of clavulanic acid did not result in an increase in the zone of inhibition.

Characterisation of AMR genes

PCR assays were carried out on all isolates suspected of ESBL production for the presence of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{AmpC} and *qnr* genes (Essack and others 2001, Pérez-Pérez and Hanson 2002, Boyd and others 2004, Robicsek and others 2006). Isolates showing resistance to ampicillin were tested for the presence of *bla*_{TEM} and *bla*_{SHV} genes. Isolates resistant to trimethoprim were examined by PCR for the presence of *dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, *dfrA9*, *dfrA12*, *dfrA13*, *dfrA14* and *dfrA17* genes (Gibreel and Sköld 1998, Lee and others 2001). In addition, the presence of *qnrA*, *qnrB* and *qnrS* genes were tested by PCR in all isolates. Finally, isolates resistant to tetracycline were screened by PCR for the following genes: *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(E)* and *tet(G)* (Ng and others 2001). Conditions and references for each PCR assay are shown in Table 1. A positive control (an isolate known to carry the gene under investigation) was included in each PCR assay.

Sequencing of *bla*_{CMY} and *bla*_{TEM} genes

For sequencing of the *bla*_{CMY} gene, oligonucleotide primers were used to amplify the entire gene (Liebana and others 2004). For *bla*_{TEM} sequencing, the amplicon was obtained using the primers for initial detection. The amplicons were cleaned using the Wizard SV gel and PCR clean-

up system (Promega), and sequenced on both strands with additional primers used for the internal sequence (two for *bla*_{CMY} sequencing and three for *bla*_{TEM} sequencing) (Mocktar and others 2008). The sequences were compared with those in GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to obtain the specific *bla*_{CMY} and *bla*_{TEM} gene variants.

Results

A total of 69 unique AMR *E coli* isolates were collected from the faeces of 53 (29 per cent, 95 per cent confidence interval [CI] 22.4 to 35.5 per cent) of the 183 dogs tested in the study. Thirteen dogs were found to be carrying more than one unique isolate of *E coli* with differing AMR profiles. The susceptibility of isolates to seven antimicrobial agents is shown in Table 2. Twenty-four per cent of dogs (95 per cent CI 17.9 to 30.2 per cent) harboured at least one isolate with ampicillin resistance, 19.7 per cent (95 per cent CI 13.9 to 25.4 per cent) with tetracycline resistance, and 16.9 per cent (95 per cent CI 11.5 to 22.4 per cent) with trimethoprim resistance. The percentage of dogs with *E coli* resistant to the other antimicrobials tested was less than 4 per cent. Twenty-seven (14.8 per cent, 95 per cent CI 10.1 to 20.5 per cent) of the 183 dogs sampled harboured at least one multidrug-resistant (MDR) (resistant to three or more antimicrobial classes) isolate. Thirty (44 per cent) of the 69 AMR *E coli* isolates were shown to be MDR and 19 (28 per cent) were resistant to two antimicrobial classes. The resistance profiles of the 69 isolates are shown in Table 3. The most common resistance profile was ampicillin-tetracycline-trimethoprim resistance, which was found in 26 per cent of the isolates, followed by ampicillin-only and tetracycline-only resistance, both of which were found in 13 per cent of isolates each, and ampicillin-trimethoprim and ampicillin-tetracycline resistance, which were found in 10 per cent of isolates each.

Only one dog, which was found not to carry AMR *E coli*, was recorded as being on a course of antimicrobials when the faecal sample was collected. Of 15 dogs reported to have had a course of antimicrobials in the previous month, five (33 per cent) carried AMR *E coli*. This was also true for nine of 37 (24 per cent) dogs whose owners reported antimicrobial use in the previous year. A similar prevalence, however, was observed among the dogs with no reported antimicrobial use.

The presence of *dfr* genes was investigated in the isolates resistant to trimethoprim; of 33 trimethoprim-resistant isolates, 11 were found to harbour *dfrA1*, eight had *dfrA5* and three had *dfrA14* genes. The remaining 11 trimethoprim-resistant isolates were negative for these genes in addition to *dfrA7*, *A8*, *A9*, *A12*, *A13* and *A17*. Of the 45 isolates resistant to tetracycline, 12 were found to harbour *tet(B)*, and none of the *tet* genes tested (*tetA*, *B*, *C*, *D*, *E* and *G*) could be detected in the remaining 33 isolates. Of the 54 ampicillin-resistant isolates, 39 were found to harbour a *bla*_{TEM} gene and none was positive for the detection of a *bla*_{SHV} gene. No *qnr* genes were detected in any of the isolates tested, including those resistant to nalidixic acid.

Results relating to the nine *E coli* isolates selected from the plates screening for ESBL production are shown in Table 4. Only one isolate was confirmed using the MAST double disc diffusion method as an ESBL-producer. A variety of resistance phenotypes were observed in these isolates, with only two sharing the same resistance profile. The nine isolates were tested for the presence of β-lactamase enzymes. A *bla*_{TEM} gene was detected in two isolates, which were identified as *bla*_{TEM-1} by sequencing. In seven isolates, *bla*_{AmpC} was detected and all were identified as the *bla*_{CMY-2} gene. No *bla*_{CTX-M} or *bla*_{SHV} genes were amplified from any of the isolates. A *bla*_{CMY-2} gene was found in the only isolate to test positive for ESBL production, but none of the other genes tested was found.

Discussion

This study aimed to estimate the prevalence of AMR *E coli* carriage in a community of healthy dogs, and found that AMR carriage was widespread in the dogs sampled (29 per cent). MDR *E coli* was also found in these dog samples (14.8 per cent). The most common antimicrobials that the isolates were resistant to were ampicillin, tetracycline and trimethoprim. This is also reflected in the most common resistance phenotype observed and might reflect the mobile nature of the genes responsible for these resistance phenotypes, and that there are numerous variants of these resistance genes.

TABLE 4: Characteristics of nine isolates recovered by ESBL screening methods from faecal samples from 183 dogs

Isolate ID	(CAZ+CA [†])-CAZ (mm)	(CPD+CA)-CPD (mm)	(CTX+CA)-CTX (mm)	Genes amplified by PCR	Resistance profile
066B(CFX)	0	0	-1	<i>bla</i> _{CMY-2} , <i>bla</i> _{TEM-1*} , <i>tet(B)</i>	AMP, LEX, CXM, FOX, CAZ, CRO, TET
084B	-5	0	-6	<i>bla</i> _{CMY-2}	AMP, AMC, LEX, CXM, FOX, CAZ, CRO
084F	0	1	-2	<i>bla</i> _{CMY-2} , <i>bla</i> _{TEM-1}	AMP, AMC, LEX, CXM, FOX, CAZ, CRO, CIP, NA, TMP, SXT, TET, CHL
091E	0	0	-1	<i>bla</i> _{CMY-2}	AMP, AMC, LEX, CXM, FOX, CRO, ATM, CIP, NA
141D	1	0	-4	None	AMP, TET
156A*	0	1	5	<i>bla</i> _{CMY-2}	AMP, AMC, LEX, CXM, FOX, CAZ, CRO, TET
157A	0	1	-5	<i>bla</i> _{CMY-2}	AMP, AMC, LEX, CXM, FOX, CAZ, CRO, TET
157C	0	0	-4	<i>bla</i> _{CMY-2}	AMP, AMC, LEX, CXM, FOX, CAZ, CRO, ATM, TET
172G	0	0	4	None	GEN, AMP, AMC, TZP, LEX, CRO

* Isolate positive for ESBL production

† Disc contained both the cephalosporin and the β-lactamase inhibitor clavulanic acid

AMC Amoxicillin-clavulanate, AMP Ampicillin, ATM Aztreonam, CAZ ceftazidime, CHL Chloramphenicol, CIP Ciprofloxacin, CRO Ceftriaxone, CXM Cefuroxime, ESBL Extended-spectrum β-lactamase, FOX Cefoxitin, GEN Gentamicin, LEX Cefalexin, NAL Nalidixic acid, SXT Trimethoprim-sulfamethoxazole, TET Tetracycline, TMP Trimethoprim, TZP Tazobactam-piperacillin

The prevalence of ampicillin resistance observed was higher than previously reported in healthy dogs in Portugal (Costa and others 2008), but similar to a study investigating, among others, healthy cat populations (Moyaert and others 2006). Resistance to other antimicrobials (amoxicillin-clavulanate, chloramphenicol, quinolones, tetracycline and trimethoprim) was comparable across all three studies. The resistances observed in the present study are much higher than reported in a study of healthy dogs in Sweden (SVA 2006), which may reflect differences in methodology. It is important to note that the dogs sampled in the present study were considered healthy based on questionnaire responses made by the owners, which may differ from other study definitions of 'healthy'. When compared with studies investigating clinical isolates, resistance to ampicillin (Normand and others 2000a) and tetracycline (De Graef and others 2004) was lower in the current population. Resistance to other antimicrobials was similar. In clinical isolates of *E. coli* from dogs in Denmark, the prevalence of AMR was comparable for all antimicrobials with the exception of nalidixic acid, which was higher in the Danish study (12.5 per cent v 3.3 per cent in the present study) (Pedersen and others 2007). Differences in the prevalence of resistance observed may be due to differences in the interpretation of the zone sizes or minimum inhibitory concentrations observed, or differences in how intermediate measurements were classified. This highlights how difficult it can be to compare different studies when a variety of methodologies and guidelines of interpretation are used and emphasises the need for more standardised methods. This may prove difficult to achieve both on an international level and between the medical and veterinary professions. However, it is likely that, compared with isolates from clinical cases, the apparently healthy household dogs sampled in the present study would have had less exposure to antimicrobials and hospital environments, both of which have been shown previously to increase the prevalence of AMR (Dunowska and others 2006, Moreno and others 2008).

The gene responsible for conferring trimethoprim resistance could be determined in only 22 of the 33 isolates tested; the genes *dfpA1*, *dfpA5* and *dfpA14* were detected and have been previously described in *E. coli* isolates of animal origin (Sáenz and others 2004, Cocchi and others 2007) and also from human isolates (Lee and others 2001). Only *tet(B)* was detected in the tetracycline-resistant isolates, which has been found in other isolates from animals (Sáenz and others 2004, Costa and others 2008, Enne and others 2008). The isolates that were negative for the genes tested are likely to harbour other genes responsible for trimethoprim and tetracycline resistance that were beyond the scope of this study and not tested.

The *qnrA*, *qnrB* and *qnrS* genes were not detected in any isolates. These genes are responsible for low-level resistance to quinolones (Martínez-Martínez and others 1998). In addition to the *qnr* genes, other mechanisms of quinolone resistance may be involved to give higher levels of resistance, for example, mutations in the genes encoding the subunits of DNA gyrase (*gyrA* and *gyrB*) or topoisomerase IV (*parC* and *parE*) (Piddock 1998).

In total, nine suspected ESBL-producing isolates were recovered. Subsequent testing confirmed only one of these to be positive for ESBL production but, as shown in Table 4, the only gene detected by

PCR was a *bla*_{CMY-2} gene. It is possible that this isolate carried a type of ESBL not tested in the present study, for example, an OXA- or VIM-type ESBL. Among the eight other isolates, two carried *bla*_{TEM-1} genes, and six carried the plasmid-mediated AmpC *bla*_{CMY-2} gene. *Bla*_{CMY} genes have previously been reported in isolates from dogs (Sidjabat and others 2006, 2007).

In summary, the present study demonstrates a common occurrence of AMR, in particular MDR, among the faecal *E. coli* of healthy dogs living in the community, with a variety of mechanisms responsible for resistance. This is of concern, given the close and frequent contact dogs have with people, and could pose a risk for the spread of resistant bacteria or resistance genes. Larger studies are required to more accurately estimate the prevalence of AMR *E. coli* in healthy dogs, and thus fully understand both the risk factors for resistance and any risks posed to people. It would also be prudent to carry out longitudinal studies to investigate whether AMR is maintained over time and how this relates to antimicrobial prescribing practices.

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