Antimicrobial-resistant *Escherichia coli* in hospitalised companion animals and their hospital environment

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**INTRODUCTION**: Antimicrobial resistance is a growing concern with implications for animal health. This study investigated the prevalence of antimicrobial resistance among commensal and environmental *Escherichia coli* isolated from animals sampled in referral hospitals in the UK.

**MATERIALS AND METHODS**: Resistant *Escherichia coli* isolated from animal faeces and practice environments were tested for susceptibility to antimicrobial agents. PCR and sequencing techniques were used to identify extended spectrum beta-lactamase and AmpC-producer genotypes.

**RESULTS**: In total, 333 faecal and 257 environmental samples were collected. Multi-drug resistant *Escherichia coli* were found in 13·1% of faecal and 8·9% of environmental samples. Extended spectrum beta-lactamase and AmpC genes were identified 14% and 7·7% of faecal samples and 8·6% and 8·6% of environmental samples, respectively. The most common extended spectrum beta-lactamase gene type detected was *bla*\(^{CTX-M−15}\), although *bla*\(^{TEM-158}\) was detected in faecal and environmental samples from one practice.

**CLINICAL SIGNIFICANCE**: *Escherichia coli* resistant to key antimicrobials were isolated from hospitalised animals and the practice environment. We identified the emergence of the inhibitor resistant and extended spectrum beta-lactamase *bla*\(^{TEM-158}\) in companion animals. Further investigation to determine risk factors for colonisation with antimicrobial-resistant bacteria is needed to provide evidence for antimicrobial stewardship and infection control programmes.


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contributed to the emergence of extended spectrum beta-
lactamase (ESBL) enzymes (Pfeifer et al. 2010), which confer
resistance to all beta-lactam antibiotics, although they remain
sensitive to beta-lactamase inhibitors, such as clavulanic acid.

E. coli can also gain resistance to beta-lactam agents by the
production of cephapemycinae AmpC enzymes which, unlike
ESBLs, also confer resistance to beta-lactamase inhibitors. In
addition, ESBL genes frequently co-exist with other resistance
genes on the same plasmid, resulting in resistance to multiple
antimicrobial classes (Woodford et al. 2004, Pirout et al. 2007,
Pirout & Laupland 2008, Schulza & Geeelings 2012). This adds to
the resistance burden within bacterial populations, because the
use of one antimicrobial class could select for resistance to mul-
tiple other classes by favouring the spread of plasmids that confer
multi-drug resistance (MDR – resistance to ≥three drug classes).

Antimicrobial-resistant E. coli have been isolated from faeces
of animals in many parts of the world. Moreover, ESBL-pro-
ducing bacteria have been associated with urinary tract infec-
tions (O’Keefe et al. 2010, Shaheen et al. 2011, Dierikx et al.
2012, Huber et al. 2013, Nebbia et al. 2014), wound infections
(Steen & Webb 2007) and cholangiohepatitis in companion
animals (Steen & Webb 2007, Timofoe et al. 2011). Among
ESBL-producing isolates from studies of companion animals
the most common types of ESBLs identified have been CTX-M
types (Carattoli et al. 2005, Sun et al. 2010, Dierikx et al. 2012,
However, data on the frequency and epidemiology of these bacte-
rria within veterinary environments in the UK are lacking.

Therefore, the aim of this study was to determine the preva-
ience of important AMR E. coli phenotypes and genotypes in the
faecal flora of hospitalised animals and the hospital environment
in referral practices in the UK. This included resistance to anti-
microbials critically important to human health (WHO 2011), such
as the third and fourth generation cephalosporins and fluoroqui-
nolones, which are also important in companion animals, as well
as resistance to commonly used antimicrobials in companion
animals, such as clavulanic acid-potentiated amoxicillin and potenti-
ated sulphonamides. In addition, we aimed to characterise and
compare the genes associated with ESBL-producing and AmpC-
producing E. coli in the environment and shed in faeces.

**MATERIALS AND METHODS**

**Practice selection and sample collection**

Five referral hospitals in Northwest UK took part in the study.
The main inclusion criterion was that the hospital must accept
secondary referral patients, including cases likely to be hospitalised.
The limitation to the Northwest region was to facilitate the sample
collection and minimise time between collection and labora-
tory processing. Written informed consent was obtained from
all owners whose animals took part in the study. Ethical approval
was granted by the University Research Ethics Committee. Sam-
ple size estimates indicated that with an expected prevalence of
ESBL-producing E. coli of 10%, 385 faecal samples would be
required to determine the prevalence with a precision of 3 and
95% confidence. Hence the aim was to collect 385 animal faecal
samples in addition to the environmental samples.

Animals eligible for the study were all dogs and cats hospitalised
for at least one night in the practice. Day cases were excluded as
were animals receiving chemotherapy or radiotherapy and those in
isolation. The target for sample collection was one faecal sample per
hospitalised animal per day. Samples were collected by practice staff,
stored in cool-boxes at the practice and collected at regular intervals.
Sampling was rotated and performed in three blocks of 2 weeks
at each practice apart from one practice that included an extra pilot
week. Environmental samples were collected by the author weekly
once during each week of sampling from each practice. There was
some variation in the exact sites selected for weekly sampling in
each practice necessitated by the different layouts. However, the
areas sampled from all practices were: ward floors; computer key-
boards in kennel rooms and treatment areas; examination tables
in treatment areas (not in consulting rooms); and the outside dog
walking areas. If there were multiple keyboards or examination
tables in the same area, one was selected on the first week of sam-
ping and was used for all subsequent sample collection. Samples
from floors were collected by wearing sterile disposable absorbent
overshoes (boot socks) moistened with 2 to 3 mL sterile saline,
walked around rooms in the same pattern each week. Samples were
taken from keyboards and tables using sterile j-clothes that are cut
to a standard size (~15 cm²), moistened with sterile saline and used
to wipe over the entire surface of keyboards and tables.

**Isolation of resistant bacteria from faecal and environmental samples**

Samples were transported to the laboratory of University of
Liverpool and processed immediately after collection. For faeces,
2 g was homogenised in brain heart infusion broth and used to
inoculate the entire surface of an eosin methylene blue agar plate
(EMBA). Antibiotic discs impregnated with ampicillin (10 µg),
clavulanic acid potentiated amoxicillin (Augmentin®; potenti-
atced amoxicillin, 30 µg), ciprofloxacin (1 µg) and trimethoprim
(2·5 µg) were added to the plate, which was then incubated over-
night at 37°C (Bartoloni et al. 2006). A 0·5-mL aliquot of the
faecal homogenate was added to 4·5-mL buffered peptone water
and incubated over night at 37°C before processing as below.

Boot socks and cloths were agitated in 20-mL buffered pep-
tone water for 2 minutes and a 10-mL aliquot incubated over-
night at 37°C. After overnight incubation environmental samples
were processed as below.

The faecal and environmental homogenates after overnight
enrichment were streaked onto 1 EMBA plate containing 1 µg/mL
ceftaxime and another containing 1 µg/mL cefazidime to isolate
cephalosporin-resistant E. coli, and incubated overnight at 37°C.

A maximum of six potential AMR E. coli isolates could be
selected per faecal sample if present: one each from the four
inhibition zones on the plate containing antimicrobial discs, one
from each of the cephalosporin-impregnated EMBA plates. Each
environmental sample yielded a maximum of two isolates if typi-
cal growth was present on the cephalosporin-impregnated plates.
All isolates were then subjected to full antimicrobial susceptibil-
ity testing as detailed below.
Antimicrobial susceptibility and ESBL phenotypic testing

Full susceptibility testing was performed on all isolates using isosensitest agar (LabM, UK) with discs impregnated with 10-µg ampicillin, 30-µg potentiated amoxicillin, 30-µg chloramphenicol, 30-µg nalidixic acid, 1-µg ciprofloxacin, 2.5-µg trimethoprim and 30-µg tetracycline, according to BSAC guidelines (BSAC 2013).

Isolates from the ceftazidime and cefotaxime EMBA plates, or which were resistant to CAPA, were also tested for ESBL phenotypes and AmpC phenotypes using the Mast Ltd (Bootle, UK) double disc set according to the manufacturer’s instructions (M’Zali et al. 2000), which has been found to have 90% sensitivity and specificity for the detection of AmpC-producer phenotypes (Ingram et al. 2011).

All antibiotic discs were obtained from Mast Ltd, the antibiotic powders from Sigma–Aldrich UK and all media from Lab M Ltd (Bury, UK).

Genotypic analysis

Cell lysates were prepared by adding two to three colonies of pure 24-hour cultures to 0.5 mL sterile water and heating at 100°C for 20 minutes. All isolates morphologically consistent with E. coli were confirmed using uidA gene primers (McDaniels et al. 1996, Maddox et al. 2011). All isolates demonstrating resistance to cefotaxime and ceftazidime on sensitivity testing were tested for bla _CTX-M _ genes using universal bla _CTX-M _ primers as previously described (Batchelor et al. 2005). To determine the CTX-M group produced, all CTX-M positive isolates were tested using primers specific to bla _CTX-M _ Groups 1, 2 (Hopkins et al. 2006) and 9 (Batchelor et al. 2005). All isolates identified as producing CTX-M group 1 ESBLs were tested further to determine whether they belonged to serogroup O25 (Clermont et al. 2008) and for markers for sequence type (ST) 131 (Clermont et al. 2009), representing the pandemic E. coli clone associated with bla _CTX-M-15 _ in human clinical infections (Lau et al. 2008).

All isolates demonstrating ceftazidime resistance on sensitivity testing were also tested for bla _TEM , bla _SHV _ and bla _OXA _ genes using a further multiplex PCR assay (Dallenne et al. 2010). All isolates demonstrating potentiated amoxicillin resistance on sensitivity testing were tested for bla _TEM _ using CITM primers (Perez-Perez & Hanson 2002). The specific genotypes of beta-lactamase genes (bla _CTX-M , bla _TEM , bla _SHV , bla _CMY ) were further determined with gene amplicons sequenced (Perez-Perez & Hanson 2002, Boyd et al. 2004, Batchelor et al. 2005) at the Zoology Sequencing Facility, University of Oxford. The specific genes were determined by comparing the sequences with those submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank).

All isolates demonstrating quinolone resistance were tested for the presence of the plasmid-mediated quinolone resistance genes, qnrA, qnrB and qnrS using a multiplex PCR assay as previously described (Robicsek et al. 2006).

Statistical analysis

Descriptive statistics and 95% confidence intervals were calculated for overall prevalence and for each practice. Resistance to each antimicrobial was considered as a separate outcome. The other outcomes considered were: MDR E. coli; resistance to third-generation cephalosporins; and ESBL- and AmpC-producer phenotypes and genotypes.

Data were clustered within veterinary practices and, because of repeated sampling, also within animal, so to obtain accurate estimates after allowing for this clustering the prevalence of AMR E. coli was estimated using multilevel, binomial models for each outcome, with practice and dog clustering accounted for by incorporation of second- and third-level random intercept terms. Calculations were performed using penalised quasi-likelihood estimates (second-order or first-order PQI). The prevalence ($P_i$) was estimated using the formula below, incorporating the constant parameter estimate ($\beta$) derived from the random intercept-only 3-level models for each outcome considered:

$$P_i = \phi_i / (1 + \phi_i)$$

95% confidence intervals for all adjusted prevalence estimates were constructed by examination of the standard errors of the intercept-only model parameters. Data were analysed using the MLwiN statistical software package (MLwiN Version 2.1 Centre for Multilevel Modelling, University of Bristol, UK).

RESULTS

A total of 333 faecal samples were collected from 214 animals over 31 sampling weeks between May 13 and October 21, 2013 (Table 1). In total 348/363 faecal isolates and 77/86

<table>
<thead>
<tr>
<th>Practice</th>
<th>Dogs</th>
<th>Cats</th>
<th>Not specified</th>
<th>Total</th>
<th>Inside floors</th>
<th>Examination tables</th>
<th>Keyboards</th>
<th>Outdoors walking area</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>122</td>
<td>14</td>
<td>0</td>
<td>136</td>
<td>28</td>
<td>12</td>
<td>6</td>
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<td>65</td>
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<td>2</td>
<td>32</td>
<td>0</td>
<td>5</td>
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<td>30</td>
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<td>6</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>10</td>
<td>0</td>
<td>73</td>
<td>24</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>0</td>
<td>2</td>
<td>36</td>
<td>24</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>0</td>
<td>6</td>
<td>51</td>
<td>30</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>298</td>
<td>24</td>
<td>13</td>
<td>333</td>
<td>136</td>
<td>36</td>
<td>54</td>
<td>31</td>
<td>257</td>
</tr>
</tbody>
</table>

*This included repeated samples from animals. The median number of samples per animal was 2 (interquartile range 1 to 3) and ranged from a minimum of 1 to a maximum of 14
†Environmental samples were collected by the author once weekly during each week of sampling, and the sites sampled were selected at the beginning of the study and remained constant. All hospitals were sampled for 6 weeks except for hospital 1 which had an additional pilot week
environmental isolates tested positive using the uidA PCR test confirming the *E. coli* identity of these isolates.

*E. coli* resistant to at least one antimicrobial were isolated from 167/333 (50.1%) faecal samples. A total of 257 environmental samples were collected during the same 31 sampling weeks (Table 1). Of these, 47 (14.3%) contained AMR *E. coli* cultured from cephalosporin selective plates.

The overall prevalence of faecal and environmental samples with at least one isolate with resistance to one or more of the six antimicrobials, to third generation cephalosporins, MDR, and ESBL-producer and AmpC-producer phenotypes, and ESBL and *bla*<sub>CITM</sub> genotypes is shown in Tables 2 and 3. AmpC resistance was the most common resistance type in both faecal and environmental samples.

There was considerable variation in the rate of isolation of resistant bacteria between practices and between areas within practices (Fig 1, and Tables 2 and 3). Practice 1 demonstrated the highest prevalence of potentiated amoxicillin resistance, ciprofloxacin resistance, and AmpC-phenotype and genotype [*bla*<sub>B</sub>ampC (CITM)] in both faecal and environmental samples. Practice 3 demonstrated the highest prevalence of ESBL-phenotype and genotype, and MDR *E. coli* among samples. Practices 2 and 5 generally demonstrated lower levels of resistance. The resistance spectrum varied among the MDR isolates from both faecal and environmental samples (Table 4). There were 18 distinct phenotypes among MDR isolates, 17 in the faecal samples and 10 in the environmental samples (although there was substantial overlap). The diversity of *E. coli* MDR phenotypes from environmental samples may have been limited due to isolation only on cephalosporin-containing media following enrichment with only two possible colony picks per sample, unlike faecal samples where up to six colony picks could be obtained. Many MDR isolates were resistant to ciprofloxacin and potentiated amoxicillin. Environmental samples associated with ESBL-, AmpC-producing, and MDR *E. coli* were most likely to be isolated from either the outside walking areas, or floors of the ward communal areas (Fig 1).

PCR detected *bla*<sub>CITM</sub> genes belonging to group 1 in *E. coli* from 22 (6-6%) faecal samples from four out of five practices and 12 (4-7%) environmental samples from three out of five practices. Group 9 *bla*<sub>CITM</sub> genes were detected in *E. coli* from 3 (0.9%) faecal samples and 2 (0.8%) environmental samples, all of which were from the same practice. No isolates were positive for CTX-M group 2, or found to belong to O25/ST131 clones by PCR assay.

The *bla*<sub>CITM</sub> gene was detected in *E. coli* from 72 (21.6%) faecal and 22 (6.6%) environmental samples, all of which were shown by sequence analysis to correspond to *bla*<sub>cmv-2</sub>. This corresponded to 81% and 69% of faecal and environmental samples with *E. coli* with potentiated amoxicillin resistance, respectively.

There was significant masking of ESBL producer phenotype by the production of AmpC with *E. coli* from 20 faecal samples and 2 environmental samples that had both AmpC and ESBL genes.

A low prevalence of *qnr* genes was found only in three (1%) faecal samples having at least one *E. coli* isolate which tested positive using the *uidA* PCR test confirming the *E. coli* identity of these isolates. The prevalence of *qnr* genes was found only in three (1%) faecal samples having at least one *E. coli* isolate which tested positive using the *uidA* PCR test confirming the *E. coli* identity of these isolates.
### Table 3. Sample level prevalence of resistance to each antimicrobial class tested in environmental isolates from cephalosporin-impregnated media (n=257) from each practice with 95% confidence intervals

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>Practice 1</th>
<th>Practice 2</th>
<th>Practice 3</th>
<th>Practice 4</th>
<th>Practice 5</th>
<th>All practices (N)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>33·8 (22·3 to 45·3)</td>
<td>6·3 (0 to 13·1)</td>
<td>25·0 (12·8 to 37·3)</td>
<td>12·5 (3·1 to 21·9)</td>
<td>6·3 (0 to 13·1)</td>
<td>46</td>
<td>17·9 (13·2 to 22·6)</td>
</tr>
<tr>
<td>CAPA</td>
<td>29·2 (18·2 to 40·3)</td>
<td>6·3 (0 to 13·1)</td>
<td>8·3 (0·5 to 16·2)</td>
<td>4·2 (0 to 9·8)</td>
<td>2·1 (0 to 6·1)</td>
<td>29</td>
<td>11·3 (7·4 to 15·2)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6·2 (0·3 to 12)</td>
<td>0·0</td>
<td>14·6 (4·6 to 24·6)</td>
<td>4·2 (0 to 9·8)</td>
<td>0·0</td>
<td>13</td>
<td>5·1 (2·4 to 7·7)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>6·2 (0·3 to 12)</td>
<td>6·3 (0 to 13·1)</td>
<td>6·3 (0 to 13·1)</td>
<td>4·2 (0 to 9·8)</td>
<td>4·2 (0 to 9·8)</td>
<td>14</td>
<td>5·4 (2·7 to 8·2)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>12·3 (4·3 to 20·3)</td>
<td>4·2 (0 to 9·8)</td>
<td>18·8 (7·7 to 29·8)</td>
<td>2·1 (0 to 6·1)</td>
<td>2·1 (0 to 6·1)</td>
<td>23</td>
<td>8·9 (5·5 to 12·4)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>29·2 (18·2 to 40·3)</td>
<td>2·1 (0 to 6·1)</td>
<td>6·3 (0 to 13·1)</td>
<td>4·2 (0 to 9·8)</td>
<td>2·1 (0 to 6·1)</td>
<td>27</td>
<td>10·5 (6·8 to 14·3)</td>
</tr>
<tr>
<td>MDR</td>
<td>15·4 (6·6 to 24·2)</td>
<td>4·2 (0 to 9·8)</td>
<td>14·6 (4·6 to 24·6)</td>
<td>4·2 (0 to 9·8)</td>
<td>4·2 (0 to 9·8)</td>
<td>23</td>
<td>8·9 (5·5 to 12·4)</td>
</tr>
<tr>
<td>Third generation cephalosporin</td>
<td>33·8 (22·3 to 45·3)</td>
<td>2·1 (0 to 6·1)</td>
<td>22·9 (11·1 to 34·8)</td>
<td>8·3 (0·5 to 16·2)</td>
<td>6·3 (0 to 13·1)</td>
<td>41</td>
<td>16·0 (11·5 to 20·4)</td>
</tr>
<tr>
<td>ESBL-producer phenotype</td>
<td>3·1 (0 to 7·3)</td>
<td>0·0</td>
<td>16·7 (6·1 to 27·2)</td>
<td>8·3 (0·5 to 16·2)</td>
<td>0·0</td>
<td>14</td>
<td>5·4 (2·7 to 8·2)</td>
</tr>
<tr>
<td>AmpC-producer phenotype</td>
<td>16·9 (7·8 to 26)</td>
<td>0·0</td>
<td>2·1 (0 to 6·1)</td>
<td>0·0</td>
<td>0·0</td>
<td>12</td>
<td>4·7 (2·1 to 7·2)</td>
</tr>
<tr>
<td>ESBL confirmed*</td>
<td>13·8 (5·4 to 22·2)</td>
<td>0·0</td>
<td>16·7 (6·1 to 27·2)</td>
<td>10·4 (1·8 to 19·1)</td>
<td>0·0</td>
<td>22</td>
<td>8·6 (5·1 to 12)</td>
</tr>
<tr>
<td>blaCTX-M positive PCR</td>
<td>23·1 (12·8 to 33·3)</td>
<td>2·1 (0 to 6·1)</td>
<td>4·2 (0 to 9·8)</td>
<td>6·3 (0 to 13·1)</td>
<td>2·1 (0 to 6·1)</td>
<td>22</td>
<td>8·6 (5·1 to 12)</td>
</tr>
</tbody>
</table>

**Note:** MDR: multidrug resistance, CAPA: clavulanic acid potentiated amoxicillin, ESBL: Extended spectrum beta-lactamase.

*Confirmed ESBL producer if either had an ESBL-producer phenotype, was positive on the universal blaCTX-M PCR or, in the case of TEM and SHV producer types, returned a sequencing result corresponding to an ESBL.

### Table 4. The different antimicrobial resistance profiles seen among the multi-drug resistance phenotypes in both faecal and environmental samples

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>Number of antimicrobial classes resistant to</th>
<th>Faecal samples</th>
<th>Environmental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of samples</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>amp aug chi trim nal cip</td>
<td>4</td>
<td>20</td>
<td>23·3 (14·3 to 32·3)</td>
</tr>
<tr>
<td>amp chi trim</td>
<td>3</td>
<td>13</td>
<td>15·1 (7·5 to 22·7)</td>
</tr>
<tr>
<td>amp aug chi tet trim nal cip</td>
<td>5</td>
<td>8</td>
<td>9·3 (3·2 to 15·4)</td>
</tr>
<tr>
<td>amp aug trim nal cip</td>
<td>3</td>
<td>7</td>
<td>8·1 (2·4 to 13·9)</td>
</tr>
<tr>
<td>amp aug chi trim</td>
<td>3</td>
<td>6</td>
<td>7·0 (1·6 to 12·4)</td>
</tr>
<tr>
<td>amp tet trim nal cip</td>
<td>5</td>
<td>5</td>
<td>5·8 (0·9 to 10·8)</td>
</tr>
<tr>
<td>amp chi tet trim nal cip</td>
<td>5</td>
<td>5</td>
<td>5·8 (0·9 to 10·8)</td>
</tr>
<tr>
<td>amp tet nal</td>
<td>3</td>
<td>4</td>
<td>4·7 (0·2 to 9·1)</td>
</tr>
<tr>
<td>amp aug tet trim nal cip</td>
<td>4</td>
<td>4</td>
<td>4·7 (0·2 to 9·1)</td>
</tr>
<tr>
<td>amp tet trim</td>
<td>3</td>
<td>3</td>
<td>3·5 (0·6 to 7·4)</td>
</tr>
<tr>
<td>amp aug tet trim</td>
<td>3</td>
<td>3</td>
<td>3·5 (0·6 to 7·4)</td>
</tr>
<tr>
<td>amp chi trim nal cip</td>
<td>3</td>
<td>1</td>
<td>1·2 (0·3 to 4·4)</td>
</tr>
<tr>
<td>amp tet cip</td>
<td>3</td>
<td>1</td>
<td>1·2 (0·3 to 4·4)</td>
</tr>
<tr>
<td>amp chi tet trim</td>
<td>5</td>
<td>5</td>
<td>1·2 (0·3 to 4·4)</td>
</tr>
<tr>
<td>amp aug chi tet trim</td>
<td>4</td>
<td>1</td>
<td>1·2 (0·3 to 4·4)</td>
</tr>
<tr>
<td>amp aug chi nal cip</td>
<td>3</td>
<td>1</td>
<td>1·2 (0·3 to 4·4)</td>
</tr>
<tr>
<td>amp chi tet trim</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

amp ampicillin resistant, aug CAPA resistant, chi chloramphenicol resistant, tet tetracycline resistant, trim trimethoprim resistant, nal nalidixic acid resistant, cip ciprofloxacin resistant
positive for qnr genes on PCR. Two were positive for qnrS and one was positive for qnrB. Among environmental samples isolates the qnrB was detected in E. coli from four (1-5%) samples.

Interestingly, the inhibitor-resistant and ESBL variant bla-TEM-158 was detected in E. coli isolated from 10 faecal samples and 1 environmental sample, all from the same practice.

Sequencing results are shown in Table 5. However, not all isolates positive for beta-lactamase genes returned sequences good enough for analysis, and these are recorded at the gene or group level only (e.g. CTX-M group 1)

DISCUSSION

Overall, in isolates from both faecal and environmental samples there were relatively high levels of resistance to a number of important antimicrobials including potentiated amoxicillin, fluoroquinolones and third generation cephalosporins. This has important implications for clinical veterinary practice and public health. Steps towards mitigating this problem will first require a thorough understanding of the prevalence and transmission in patients and the practice environment.

Table 5. Number and percentage of faecal (n=333) and environmental (n=257) samples with at least one Escherichia coli isolate having a resistance gene identified by sequence analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Faecal samples</th>
<th>Environmental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaCTX-M-1</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>blaCTX-M-12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>blaCTX-M-9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>blaCTX-M-82</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>bliaM-158</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>blaTEM-158</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>blaCMY-2</td>
<td>72</td>
<td>22</td>
</tr>
</tbody>
</table>

*In five isolates positive on the universal blaTEM-PCR the group was not identified

There was frequent potentiated-amoxicillin resistance among samples from all the practices, with an adjusted prevalence of 14% (albeit with some variation between practices). Potentiated amoxicillin is commonly used in companion animal practice in the UK (Mateus et al. 2011, Radford et al. 2011) and the frequent carriage of resistance is likely related to the frequency of use. Nevertheless, this is of some concern. In human medicine the prevalence of ESBLs in hospitals has been linked to use of potentiated amoxicillin in the community, highlighting the importance of interaction between community antimicrobial use and the development of AMR in hospitals (Aldeyab et al. 2012). Resistance to potentiated amoxicillin has been found in dogs in other studies, but at lower levels ranging from 6-3 to 8-3%. The high levels in this study are likely to reflect hospitalisation, because a study of non-vet visiting dogs in a similar area showed a relatively low level of potentiated amoxicillin resistance (Wedley et al. 2011).

The majority of E. coli with potentiated-amoxicillin resistance tested positive for blaAmpC genes, making AmpC production the most likely mechanism responsible for resistance. AmpC genes conferring AmpC production have also been found in clinical E. coli isolates from animals in the USA (Shaheen et al. 2011) and the Netherlands (Dierikx et al. 2012), and in hospitalised animals in Korea (So et al. 2012) and Australia (Sidjabat et al. 2006). High levels of AmpC-production have been found in a human healthcare setting where they were putatively linked with a high use of potentiated-amoxicillin in the same facility (Seiffert et al. 2013). These results suggest that AmpC-production is potentially an important mechanism conferring potentiated-amoxicillin resistance among hospitalised companion animals.

Potentiated-amoxicillin resistance and AmpC genes were found in environmental samples from all practices, although there was variation between practices, which followed a similar pattern to that seen in the faecal samples, suggesting a link between commensal faecal and environmental-resistant E. coli. Colonisation of dogs and humans and contamination of the veterinary environment by the same AmpC-producing E. coli strains has been reported previously (Sidjabat et al. 2006) and the environmental prevalence (4-1%) reported is similar to levels found in most of the practices in this study.
The prevalence of resistance to ciprofloxacin (a marker for fluoroquinolone resistance) was variable between practices, ranging from 8% to 44%. In contrast, ciprofloxacin resistance was only found in 2-2% of community dogs using similar methods in the same area (Wedley et al. 2011). Moreover, 73% of the 80 faecal samples with ciprofloxacin-resistant E. coli demonstrated MDR phenotypes. Fluoroquinolones are a class of antimicrobial used in treatment of important infections in both human and veterinary medicine and are classed as a high priority antimicrobial (WHO 2011), and resistance to these drugs is a serious concern for animal and public health. The frequent co-association of fluoroquinolone resistance with resistance to other, unrelated classes of antimicrobial is an additional concern. The qnr genes were detected at low rates among both faecal and environmental samples and are therefore unlikely to be a significant contributor to the levels of quinolone resistance observed in this study. Further mechanisms for quinolone resistance in this study were not studied, but they are most likely to be due to chromosomal mutations in the gyrA genes.

The prevalence of MDR in faecal samples from this study was 13%. The similar resistance profiles between MDR faecal and environmental samples are suggestive of cross contamination between animals and their environment. Of particular concern is the relatively frequent isolation of bacteria resistant to all seven antimicrobials tested. Other studies reported MDR in 48% in hospitalised dogs and 32% of stray dogs from Korea (Nam et al. 2010), 15% of community-based dogs in the UK (Wedley et al. 2011) and 9% of animals at admission in the USA (Hamilton et al. 2013). It has also been shown that resistant organisms are more prevalent among hospitalised or sick animals compared to non-hospitalised or healthy animals (Nam et al. 2010, Sun et al. 2010, Gibson et al. 2011). It is also noteworthy that the MDR isolates in this study frequently included resistance to potentiated amoxicillin and fluoroquinolones, whereas this was uncommon in MDR isolates from community-based dogs (Wedley et al. 2011), which were most frequently resistant to ampicillin, tetracycline and trimethoprim.

Resistance to third generation cephalosporins was detected at relatively high rates in this study. Studies of companion animals have found variable levels of resistance depending on setting and methods used: ranging from 60-5% in hospitalised pets treated with antimicrobials in China (Lei et al. 2010); 13% in dogs and cats from the community and nursing homes in Switzerland (Gandolfi-Decristophoris et al. 2013); and 2-4% from stray and hospitalised dogs in Korea (Nam et al. 2010).

The confirmed sample prevalence of ESBL-producer phenotypes in this study was 13%. ESBL production has been detected in: 54-5 and 24-5% in sick and healthy animals, respectively, in veterinary hospitals in China (Sun et al. 2010); 33-3% in Korean veterinary hospitals (So et al. 2012); 5% of faecal samples from cats and dogs in shelters in Germany (Franiek et al. 2012); and 16% of faecal samples from healthy cats and dogs in Tunisia (Ben Sallem et al. 2013). ESBLs have also been detected from clinical urinary isolates in the USA (O’Keefe et al. 2010, Shaheen et al. 2011) and Switzerland (Huber et al. 2013). There is clearly variation in ESBL-producer prevalence by location and setting. Hospitalised animals appear to be at greater risk, though this study demonstrated variation among hospitals and there are likely to be hospital level factors that have an important influence. Assessing ESBL production by phenotypic results only led to an underestimation of the true prevalence in this study due to masking by AmpC-producer phenotypes in the same isolates. This was particularly of note in the practice that had high levels of AmpC production, demonstrating the importance of production of AmpC not only in the resistance it confers but also in its ability to make detecting and confirming ESBL production more difficult.

The most common ESBL genotype identified in this study was \(\text{bla}_{\text{CTX-M-15}}\). Several other studies have found CTX-M-1 production to be more common in companion animals isolates (Costa et al. 2008, Dieriks et al. 2012). However, others have identified \(\text{bla}_{\text{CTX-M-15}}\) in companion animals (Sun et al. 2010, Dieriks et al. 2012, So et al. 2012, Huber et al. 2013, Belas et al. 2014) and in Europe it is the second most commonly isolated ESBL gene from companion animals (Ewers et al. 2012). A number of ESBL-positive samples in this study were found to carry \(\text{bla}_{\text{CTX-M-15}}\) genes, but these could not be assigned to a group (n=5 E. coli) or, when assigned to a group, the sequence was poor so that it could not be used to determine the specific \(\text{bla}_{\text{CTX-M}}\) gene type.

The true prevalence of CTX-M type ESBLs may therefore be different. The \(\text{bla}_{\text{CTX-M-15}}\) gene is the most common gene associated with the human pandemic O25/ST131 E. coli clone in the UK (Lau et al. 2008); however, no E. coli isolates that carried \(\text{bla}_{\text{CTX-M}}\) genes in this study were positive for markers for this clone. However, other E. coli clones have been isolated from companion animals in Europe associated with CTX-M production, such as ST648 (Ewers et al. 2014). The \(\text{bla}_{\text{TEM-158}}\) gene was the second most prevalent ESBL genotype found in this study. It was first detected from a faecal sample from a human intensive care patient in France and demonstrates both ESBL and inhibitor resistant TEM (IRT) characteristics (Robin et al. 2007), and due to this is often referred to as a complex mutant TEM (CMT). Other studies have identified TEM-158 production in urinary E. coli isolates in the human community in Morocco (Barguiguia et al. 2013) and clinical isolates from human patients in Kenya (Kitu et al. 2012). Evolution of TEM-158 has been shown to occur as a result of antimicrobial therapy in humans (Jacquier et al. 2013). Occurrence of this beta-lactamase at relatively high levels in one hospital is of concern given that it confers a wider spectrum of resistance to beta-lactams and the fact that its inhibitor resistant characteristics can make identification difficult. Molecular methods including sequencing are required to distinguish TEM-158 production from that of AmpC and other ESBL variants. We are not aware of any other studies in companion animals that have identified the presence of \(\text{bla}_{\text{TEM-158}}\). As the only studies reporting this previously were from humans it may be that inter-species transmission has occurred or, alternatively, it may have been driven by antimicrobial therapy. Further study is needed to determine factors responsible for this, including examining the referring population of animals in the community.

Contamination of the human hospital environment with ESBL-producing Enterobacteriaceae originating from patients

has been reported (Guet-Revillet et al. 2012). Furthermore, in a companion animal veterinary hospital in Canada environmen-
tal contamination with *E. coli* was detected in 92% and CMY-2
(AmpC) producing *E. coli* in 9% of the hospitals sampled (Mur-
phy et al. 2010). These findings suggest that *E. coli* with im-
portant resistance phenotypes such as ESBL and AmpC producers,
are present in practice environments, where they may act as a
source of infection and reservoir of resistance determinants.

For most practices the outside walking and toileting area were
the areas with the most frequent isolation of resistant bacteria, despite practice staff picking up and disposing of faeces. Con-
tamination of this area with *E. coli* may be associated with large
numbers of dogs, mixing from different wards and difficulty with
disinfection of the surface. Practices 1, 2 and 5 had grassy walk-
ing areas and practice 4 had a wood chip surface, which all would
be difficult to disinfect. Only practice 3 had a concrete surface
that should be easier to disinfect, and the isolation rate from the
outside area in this practice was similar to other areas within the
practice. Internal floors were next most frequently associated with
resistant bacteria, with tables and keyboards less commonly con-
taminated. This may be associated with effective hand hygiene
and/or the relative ease of cleaning and disinfecting these sites.

One weakness of this study was the variable number of sam-
pies returned by practices because of low throughput of animals and
a lack of hospitalised patients in some hospitals. In addition,
for ethical reasons, only naturally-voided faecal samples were
collected, and animals that did not defecate (e.g. when hospi-
talised for short periods or if non-ambulatory) may be under-
represented. There was also some variation in time between
sample collection and processing, although this never exceeded
3 days. The use of bags to collect faeces may have led to under-
representation of soft or liquid faeces, therefore diarrhoeic dogs
may also be underrepresented. Cats were included as there have
been no equivalent studies of their carriage of resistant bacteria,
but the study power was limited by low numbers associated with
less frequent hospitalisation of cats. Environmental sample col-
collection was variable as this was designed to fit around practice
routine to minimise disruption. It is therefore possible that clean-
ing may have occurred at variable times before sample collection,
which could have affected isolation rates of *E. coli*. Furthermore,
only potential ESBL/AmpC producers were isolated following
enrichment of environmental samples. Therefore, cephalospo-
rin isolation rates and phenotypes are only directly comparable
between faecal and environmental samples. Our study also used
methodology which specifically selected for resistant variants of
*E. coli*, so our reported prevalence may be higher compared to other studies in which selective isolation was not employed.

Nevertheless because of this our findings are likely to reflect the
true prevalence concerning faecal carriage of resistant bacteria in
hospitalised animals. Finally, this study was based in secondary
to tertiary referral hospitals, and the results may not reflect first
opinion practice.

In summary, this project demonstrated that important AMR,
MDR and ESBL phenotypes and genotypes are present among
faecal and environmental *E. coli* in veterinary hospitals, includ-
ing the detection of production of the IRT TEM-158. Such *E.
coli* are potential pathogens (Steen & Webb 2007, O’Keeffe et al.
2010, Timofte et al. 2011, Huber et al. 2013, Nebbia et al. 2014),
and treatment options for infections would be limited. Moreover,
close contact between pets and their owners (Westgarth et al.
2008) could result in zoonotic transmission of resistant organisms
or resistance determinants if present on mobile genetic elements.
Contamination of the practice environment with these bacteria is
of concern because environmental bacteria could be dissemi-
nated to new sites and animals (particularly vulnerable patients).
They may also act as a source of infection for new animals in the
environment and a source of resistance genes for exchange with
previously susceptible *E. coli*. Further work is required to study
the transmission of such resistant bacteria in companion animals
and their practice environment, the resistance determinants and
their mobility, and their association with virulent clones, to show
how this contributes to clinical infections with resistant organ-
isms. Furthermore determining risk factors associated with envi-
ronmental contamination and carriage of resistant commensal
*E. coli* and elucidating reasons for the differences between practices
will help inform effective infection control measures and antimi-
crobal stewardship programmes.

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Conflict of interest

None of the authors of this article has a financial or personal
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