

# Veterinary Hospital Dissemination of CTX-M-15 Extended-Spectrum Beta-Lactamase–Producing *Escherichia coli* ST410 in the United Kingdom

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We characterized extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated quinolone resistance (PMQR) in 32 *Escherichia coli* extended spectrum cephalosporin (ESC)-resistant clinical isolates from UK companion animals from several clinics. In addition, to investigate the possible dissemination of ESBL clinical isolates within a veterinary hospital, two ESBL-producing *E. coli* isolates from a dog with septic peritonitis and a cluster of environmental ESC-resistant *E. coli* isolates obtained from the same clinic and during the same time period, as these two particular ESBL-positive clinical isolates, were also included in the study. Molecular characterization identified *bla*<sub>CTX-M</sub> to be the most prevalent gene in ESC-resistant isolates, where 66% and 27% of clinical isolates carried *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub>, respectively. The only PMQR gene detected was *aac(6′)-Ib-cr*, being found in 34% of the ESC *E. coli* isolates and was associated with the carriage of *bla*<sub>CTX-M-15</sub>. The clinical and environmental isolates investigated for hospital dissemination had a common ESBL/AmpC phenotype, carried *bla*<sub>CTX-M-15</sub>, and co-harbored *bla*<sub>OXA-1</sub>, *bla*<sub>TEM-1</sub>, *bla*<sub>CMY-2</sub>, and *aac(6′)-Ib-cr*. Multilocus sequence typing identified them all as ST410, while pulse-field gel electrophoresis demonstrated 100% homology of clinical and environmental isolates, suggesting hospital environmental dissemination of CTX-M-15–producing *E. coli* ST410.

**Keywords:** *E. coli*, ESBL, surveillance, veterinary, infection control

## Introduction

*ESCHERICHIA COLI* ARE opportunistic pathogens in humans and companion animals and can be associated with a variety of extraintestinal infections, which may require antimicrobial therapy.<sup>1</sup> Increased use of antimicrobials in companion animals may select for antimicrobial-resistant bacteria, and concerns have been raised that these host species may act as potential reservoirs for human infections.<sup>2</sup> Particularly concerning for both human and veterinary health is the increasing resistance to extended spectrum cephalosporins (ESCs) through the production of extended-spectrum beta-lactamases (ESBLs).<sup>3–6</sup> There is increasing evidence that *E. coli*-producing ESBL and/or AmpC beta-lactamases are emerging in companion animals,<sup>7,8</sup> setting new challenges for veterinary practitioners due to therapeutic and infection control implications.

Several studies have described the prevalence of ESBL resistance in bacteria from companion animals,<sup>3,4,9–11</sup> but the

role that these animals may play in the spread of such resistant bacteria or determinants, is not yet fully determined. Previous molecular studies have shown that, in human hospital settings, ESBL genetic determinants have the potential to spread either through clonal dissemination or plasmid transfer, posing a serious threat to patient care and safety.<sup>6,12</sup> The development of large veterinary hospitals with intensive care facilities has created similar conditions for the emergence of animal hospital acquired infections and a few studies have shown the association of multidrug resistant (MDR) organisms, such as *E. coli*, *Acinetobacter baumannii*, *Enterobacter* spp., and *Enterococcus* spp., with animal nosocomial infections.<sup>13–19</sup> However, with the exception of a few recent studies showing the hospital acquisition and/or dissemination of beta-lactamases or ESBL-producing *Klebsiella pneumoniae* and *A. baumannii*<sup>20–23</sup> and compared with the wealth of data from human medicine, there is a paucity of studies investigating the potential of ESBL-producing *E. coli* to spread and cause nosocomial infections in veterinary clinics or hospital settings.

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The aim of this study was dual; first, to characterize ESBL and plasmid-mediated quinolone resistance (PMQR) genes in *E. coli* from clinical specimens submitted for routine bacterial culture to the Veterinary Microbiology Diagnostics laboratory in the Liverpool School of Veterinary Science. Second, to analyze and compare a cluster of clinical and environmental ESBL-producing *E. coli* obtained from a UK Veterinary Hospital to identify the potential of clinical isolates to spread within such environments.

## Materials and Methods

### Bacterial isolates

All *E. coli* isolates were obtained from companion animal clinical specimens submitted from a veterinary hospital and a number of small veterinary clinics and collected for this study between January 2010 and November 2011. Clinical specimens were plated out aerobically on 5% sheep blood agar (Oxoid) and incubated for 24 hours at 37°C. Clinical isolates presumptively identified as *E. coli* based on a positive reaction on Eosin Methylene Blue Agar (EMBA; Oxoid, Basingstoke, UK) and which showed reduced susceptibility to cefpodoxime (10 µg) and/or cefoxitin (30 µg), used as indicators for ESBL and AmpC production, were selected for this study. In addition, samples from active bacterial environmental surveillance, which is also offered for veterinary hospitals as part of the Diagnostic Service, are also processed by the laboratory, and a cluster of hospital environmental *E. coli* isolates was also included in this study. Detection of resistance to ESC in environmental *E. coli* isolates followed the same protocols as for clinical isolates. The identification of clinical and environmental isolates was performed using API 20E Identification Kits (bioMérieux, France) and also by PCR detection of the *uidA* gene for confirmation of *E. coli*.<sup>24</sup>

### Antimicrobial susceptibility testing

Susceptibility testing was performed by disc diffusion to representatives of beta-lactam and non-beta-lactam antimicrobial classes on ISO-Sensitest agar (Oxoid, Basingstoke, UK) and results were interpreted according to the BSAC (British Society for Antimicrobial Chemotherapy) interpretative criteria.<sup>25</sup> *E. coli* ATCC 25922 was used as control strain. All isolates were tested for ESBL production by the double disc synergy test (DDST).<sup>26</sup>

### Characterization of ESBL and other resistance genes

Cell lysates obtained from all investigated isolates were screened by PCR and DNA sequencing for the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>OXA</sub>, plasmid-mediated *bla*<sub>AmpC</sub> variants, PMQR genes *qnrA*, *B*, *S*, as well as the *cr* variant of *aac(6)-Ib*, as previously described.<sup>27-31</sup> Specific PCR assays were performed to identify the possible association of *bla*<sub>CTX-M-15</sub> with *ISEcp1* or *IS26* insertion elements, which have been shown to be involved in the mobilization and expression of *bla*<sub>CTX-M</sub> genes.<sup>32,33</sup>

### Resistance transfer and PCR-based replicon typing

To determine the transferability of the ESBL and PMQR genes, conjugation by plate mating was performed with streptomycin-resistant *E. coli* HB101 as the recipient and se-

ven selected donors harboring *bla*<sub>CTX-M-15</sub> ( $n=6$ ) or *bla*<sub>CTX-M-14</sub> ( $n=1$ ). Plasmid replicons involved in the transfer of the resistance genes were analyzed by PCR-based plasmid replicon typing (PBRT) as described by Carattoli *et al.*<sup>34</sup>

### Molecular characterization of isolates

A multiplex PCR described by *Clemont et al.*,<sup>35</sup> was used to assign the *E. coli* isolates to a phylogenetic group. Genetic relatedness of isolates identified to carry *bla*<sub>CTX-M</sub> genes was analyzed by macrorestriction pulsed-field gel electrophoresis (PFGE) ([www.cdc.gov/pulsenet/pathogens/](http://www.cdc.gov/pulsenet/pathogens/)). Data were analyzed using BioNumerics software version 5.1 (Applied Maths). A tolerance of 1.00% was selected and cluster analysis of PFGE pulsotypes was performed by the unweighted pair group method with average linkages (UPGMA), using the Dice coefficient to analyze similarities and define pulsotypes. PFGE pulsotypes were identified as isolates with ≥90% similarity. Multilocus sequence typing (MLST) was performed as previously described<sup>36</sup> for at least one isolate from each identified PFGE cluster.

## Results

### Bacterial isolates and antimicrobial susceptibility testing

Four hundred and forty five *E. coli* isolates ( $n=445$ ) were obtained from companion animal clinical specimens between January 2010 and November 2011, of which 32 (7%) cefpodoxime and/or cefoxitin-resistant nonduplicate isolates (30 canine and two feline) were characterized in this study. The selected isolates were both from normally sterile sites [urine ( $n=6$ ), liver/bile ( $n=4$ ), abdominal fluid ( $n=3$ ), bronchoalveolar lavage ( $n=1$ ), lymph-node biopsy ( $n=1$ )] and also from sites colonized with normal flora and where cultures yielded mixed bacterial growth [colon biopsies ( $n=3$ ), wounds ( $n=4$ ), skin/ear swabs ( $n=5$ ), fecal samples ( $n=5$ )]. In addition, a cluster of environmental ESC-resistant *E. coli* isolates ( $n=6$ ) obtained from the same clinic and during the same time period as two particular ESBL-positive clinical isolates, was also included in the study and characterized by the same methods as the clinical isolates. These two ESBL-positive clinical isolates were from the same dog, which had been admitted with septic peritonitis following duodenal ulceration; one isolate was obtained from abdominal fluid (12L-0659) and one from a surgical site wound swab (12L-0671) following surgery. The environmental ESC-resistant *E. coli* isolates were obtained from the ultrasound table (EBM-111) where the dog was examined and also from various areas of the ward where the dog was hospitalized; these included the kennel area, the drip pump attached to the kennel, the ward door handle, the ward fridge handle, and the ward computer keyboard (EBM-114, EBM-115, EBM-116, EBM-118, and EBM-119).

All isolates characterized in this study showed resistance to ampicillin, amoxicillin-clavulanic acid (CV), cefotaxime, cefpodoxime, ceftazidime, and tetracycline. In addition, 71% of isolates exhibited resistance to cefoxitin, 65% to ciprofloxacin, and 59% to trimethoprim/sulfamethoxazole (Table 1). Interestingly, 29% of isolates showed resistance to amoxicillin-clavulanic acid, but susceptibility to cefoxitin.

TABLE 1. PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF ESC-RESISTANT FELINE AND CANINE *ESCHERICHIA COLI* CLINICAL AND ENVIRONMENTAL ISOLATES (GROUPINGS BASED ON COMMON GENE COMBINATIONS, PG, AND ST, WERE DETERMINED)

Number of isolates (n = 38)	Species	Isolate ID	Source	Common antimicrobial resistance profile	IS type	Beta-lactamase genes	PMQR genes	PG	ST	PT
n = 9	Dog	11L-2603 12L-0659 12L-0671 EBM (111, 114, 115, 116, 118, 119) 11L-1050A	Colon biopsy Wound Abdominal fluid Hospital environment Liver biopsy	AMP, AMC, CPD, CTX, CAZ, FOX, CIP, NA, CN, TE, STX	ISE <i>epI</i> disrupted by IS26	<i>bla</i> <sub>CTX-M-15</sub> , OXA-1, TEM-1, CMY-2	<i>aac-6Ib-cr</i>	A	410	4 4 4 4 (x6)
n = 1	Dog	11L-1050A	Liver biopsy	AMP, AMC, CPD, CTX, CAZ, FOX, CIP, NA, CN, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA-1, TEM-1	<i>aac-6Ib-cr</i>	D	2348	7
n = 2	Dog	10L-3852 10L-3690	Feces Skin swab	AMP, AMC, CPD, CTX, CAZ, FOX, NA, CIP, CN, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA-1	<i>aac-6Ib-cr</i>	A		5 4
n = 3	Dog	10L-4543 11L-1298 10L-2646 11L-0348	Skin swab Skin swab Bile Colon biopsy Ear swab	AMP, AMC, CPD, CTX, CAZ, CIP, NA, CN, TE, S, STX	IS26 (400bp)	<i>bla</i> <sub>CTX-M-15</sub> , OXA-1	<i>aac-6Ib-cr</i>	B2	131	6 6 6 6
n = 1	Dog	11L-4755	Feces	AMP, AMC, CPD, CTX, CAZ, FOX, CIP, NA, CN, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA	<i>aac-6Ib-cr</i>	D	2348	8
n = 1	Dog	10L-1340	Feces	AMP, AMC, CPD, CTX, CAZ, FOX, CIP, NA, CN, TE, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA	<i>aac-6Ib-cr</i>	A	1284	9
n = 1	Dog	10L-0827	Abdominal fluid	AMP, CPD, NA, CIP, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA		A	4184	1
n = 4	Dog	10L-0405/ 10L-0652 10L-0784(A) 10L-0784(B)	LN biopsy Urine Bile Bile	AMP, AMC, CPD, CTX, CAZ, FOX, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA		A	4184	1
n = 1	Dog	11L-2596	Urine	AMP, CPD, NA, CIP, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA		A	4184	1
n = 3	Dog	10L-1747/ 10L-2253/ 11L-2520 11L-1050B	Urine Urine Skin swab BAL	AMP, AMC, CPD, CTX, CAZ, FOX, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>CTX-M-15</sub> , OXA		A	4184	1
n = 2	Dog	11L-1345/ 10L-4304	Abdominal fluid Urine	AMP, AMC, CPD, CTX, CAZ, FOX, CIP, NA, CN, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>TEM-1</sub> , CMY-2		D		
n = 1	Dog	12L-0098	Urine	AMP, AMC, CPD, CTX, CAZ, FOX, TE, S, STX	ISE <i>epI</i>	<i>bla</i> <sub>TEM-1</sub> , CMY-2		A		
n = 1	Dog	10L-4532	Feces	AMP, AMC, CPD, CTX, CAZ, FOX, TE, S	ISE <i>epI</i>	<i>bla</i> <sub>TEM-1b</sub> , CMY-2		B2		
n = 3	Dog	10L-4885/ 11L-0024 12L-0372	Urine Wound infection Feces	AMP, AMC, CPD, CTX, CAZ, FOX, NA, TE	ISE <i>epI</i>	<i>bla</i> <sub>TEM-1</sub> , CMY-2		A		
n = 1	Dog	10L-3142	Swab	AMP, AMC, CPD, CTX, CAZ, FOX, TE	ISE <i>epI</i>	<i>bla</i> <sub>TEM-1</sub> , CMY-2		B2		
n = 2	Feline	11L-0677/ 10L-2129	Wound infection Wound infection	AMP, AMC, CPD, FOX, TE, S	ISE <i>epI</i>	<i>bla</i> <sub>CMY-2</sub>		B2 B2		

The numbers of isolates with a common phenotype and genotype are shown in column 1.

EBM, environmental bacterial monitoring; BAL, bronchoalveolar lavage; LN, lymph node; PMQR, plasmid-mediated quinolone resistance; PG, phylogenetic group; ST, sequence type; PT, pulsotype; AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CFP, cefpodoxime; CTX, cefotaxime; CAZ, ceftazidime, FOX, cefoxitin; CIP, ciprofloxacin; NA, nalidixic acid; CN, gentamicin; TE, tetracycline; S, streptomycin, STX, trimethoprim/sulfamethoxazole.

This may indicate that mechanisms such as those which involve combinations *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-1</sub> genes (as seen in isolates 10L-4543, 11L-1298, 10L-2646) may give rise to this phenotype. Other mechanisms responsible for amoxicillin-clavulanic acid resistance, but which do not normally confer resistance to the cephamycins includes hyperproduction of TEM-1 or SHV-1 beta-lactamases. Although we did not attempt to determine whether this was the case, a number of the tested isolates carried *bla*<sub>TEM-1b</sub> only and were fully susceptible to ceftaxime (10L-1747, 10L-2253, 11L-2520, Table 1).

The eight *E. coli* isolates included in this study for comparison (two clinical and six environmental) were processed in the diagnostic laboratory simultaneously and the identical susceptibility phenotypes identified in this group of isolates triggered closer investigation. In the DDST, they showed no synergy for ceftazidime and cefotaxime with CV combinations, while only a small zone of inhibition (less than 4 mm) appeared for the cefpodoxime/CV combination. All isolates were resistant to ceftaxime and this raised the possibility of the ESBL phenotype being masked by the additional presence of AmpC cephalosporinase, which is not inhibited by CV; additional testing with a cefepime/CV combination revealed the presence of ESBL phenotypes in these eight clinical/environmental *E. coli* isolates.

#### Characterization of ESBL and other resistance genes

Among the ESC-resistant clinical *E. coli* isolates, CTX-M type ESBL was the most prevalent, found in 56% (18/32) of isolates, of which 66% (12/18) of isolates harbored *bla*<sub>CTX-M-15</sub>, with *bla*<sub>CTX-M-14</sub> being found in five isolates (27%) and *bla*<sub>CTX-M-27</sub> identified in one isolate. With the exception of one isolate, which carried *bla*<sub>CTX-M-15</sub> alone, the remaining ESC-resistant clinical *E. coli* isolates also carried *bla*<sub>TEM-1</sub>, *bla*<sub>CMY-2</sub>, and/or *bla*<sub>OXA-1</sub> in various combinations (Table 1). The *aac*(6')-Ib-cr gene was the only PMQR gene detected, although at high prevalence (34.3%), and was associated with the carriage of *bla*<sub>CTX-M-15</sub>. The eight clinical and environmental isolates that showed the common ESBL/AmpC phenotype, carried *bla*<sub>CTX-M-15</sub> and also co-harbored *bla*<sub>OXA-1</sub>, *bla*<sub>TEM-1</sub>, *bla*<sub>CMY-2</sub>, and *aac*(6')-Ib-cr. Specific PCR assays revealed that *ISEcp1* or IS26, or in some isolates *ISEcp1* disrupted by IS26, was associated with *bla*<sub>CTX-M-15</sub> (Table 1). In addition, *ISEcp1* was associated with *bla*<sub>CTX-M-14</sub> in one of the five isolates and was not found to be associated with *bla*<sub>CTX-M-27</sub>, findings which support the diversity of the CTX-M genetic arrangements in *E. coli* isolates resulting from various mobilization events.

#### Resistance transfer and PBRT

Five transconjugants (four *bla*<sub>CTX-M-15</sub> and one *bla*<sub>CTX-M-14</sub>), for which the ESBL phenotype and the presence of *bla*<sub>CTX-M-14/15</sub> was confirmed, were generated on nutrient agar supplemented with cefotaxime (1 mg/L) and streptomycin (50 mg/L). PBRT showed that the transfer of *bla*<sub>CTX-M-15</sub> was mainly associated with the FIA (*n*=4), FIB (*n*=4), IncI1 (*n*=3), Y (*n*=2), and B/O (*n*=1) replicon types, while IncY type replicon was associated with the transfer of *bla*<sub>CTX-M-14</sub>. PCR also showed that *bla*<sub>TEM-1</sub>, *bla*<sub>CMY-2</sub>, *bla*<sub>OXA-1</sub>, as well as *aac*(6')-Ib-cr, had cotransferred with the

*bla*<sub>CTX-M-14/15</sub> in all transconjugants, indicating that they are located on conjugative plasmids.

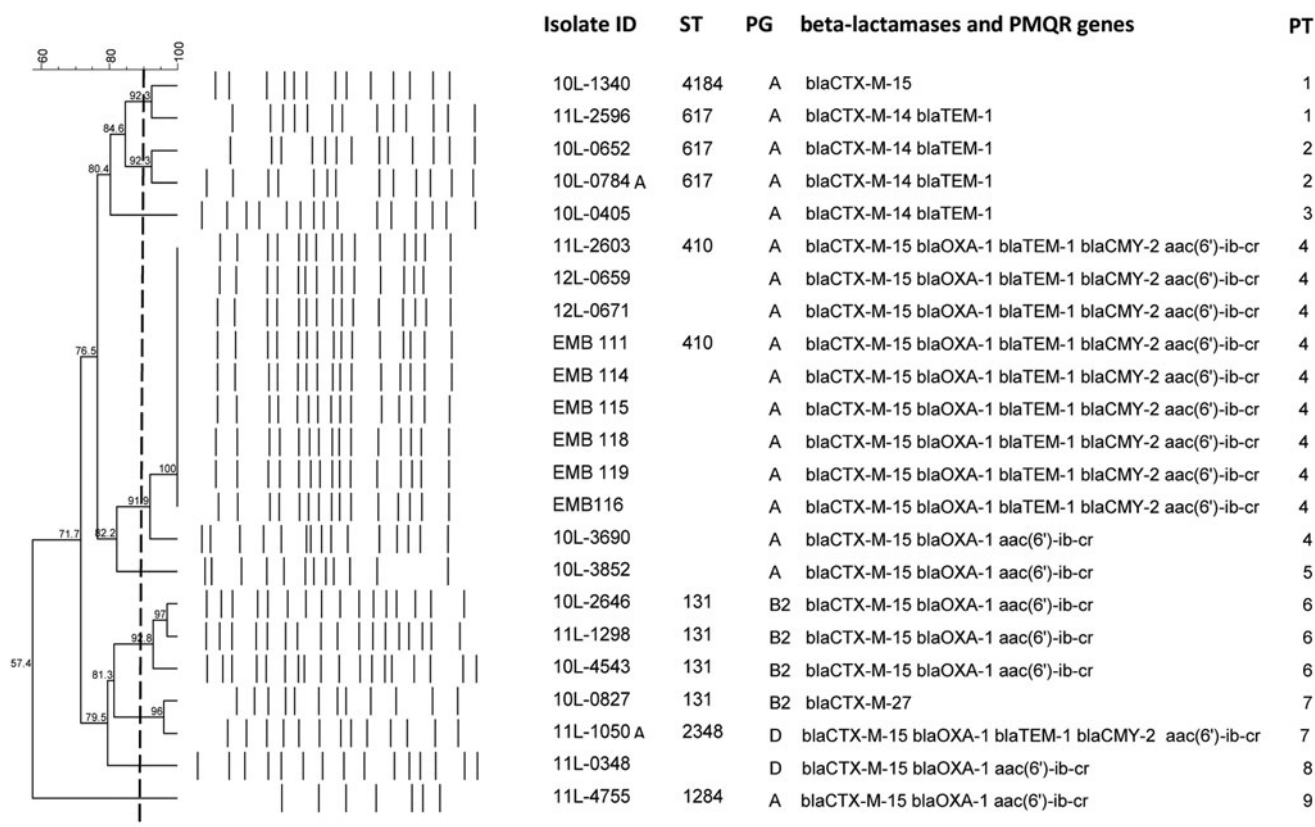
#### Molecular typing of isolates

Phylogenetic typing identified that 63% of *E. coli* isolates belonged to phylogenetic group A and the remaining isolates were typed to the more potentially pathogenic groups, B2 (21%) or group D (15%). PFGE showed clonal diversity of the CTX-M-positive isolates and five pulsotypes (PT 1, 2, 4, 6, and 7) were identified with similarity of isolates greater than 90% (Fig. 1). The main group (PT 4, *n*=9) included the eight clinical and environmental isolates with the common ESBL/AmpC phenotype and interestingly, another clinical isolate from a colon biopsy obtained from a dog (11L-2603), which was admitted with diarrhea in the same clinic 7 months previously. The second main cluster (PT 6) was formed by three isolates identified to belong to the human pandemic ST131 by MLST. Interestingly, a fourth member of this clone, which carried *bla*<sub>CTX-M-27</sub>, showed only a 73% similarity with the ST131 group. MLST also showed that the clinical and environmental isolates with the common ESBL/AmpC phenotype belonged to ST410, while the next most common ST identified in our *bla*<sub>CTX-M</sub> isolates was ST617 (Fig. 1).

#### Discussion

This study characterized a collection of ESC-resistant *E. coli* and identified a high prevalence (7%) of ESC-resistant *E. coli* in clinical specimens from companion animals in the UK, which is considerably higher than that found in similar studies from pets in France (3.7%) or The Netherlands (2%).<sup>3,10</sup> We also found a high prevalence (56%) of CTX-M type ESBL-producing *E. coli* from clinical specimens where 66% of clinical ESBL-producing *E. coli* carried *bla*<sub>CTX-M-15</sub>, which is among the highest reported rates in companion animals. To the best of our knowledge, higher carriage rates of *bla*<sub>CTX-M-15</sub> in clinical animal isolates have only been reported in the United States where 78% of the ESBL-producing *E. coli* clinical isolates from companion animals were found to carry *bla*<sub>CTX-M-15</sub>.<sup>11</sup> In Europe, 46% and 36% of canine ESBL-producing *E. coli* isolates (from Germany and France, respectively) carried *bla*<sub>CTX-M-15</sub>.<sup>10,37</sup> In The Netherlands, Dierikx *et al.*,<sup>3</sup> found that of 29 *E. coli* isolates with an ESBL/AmpC phenotype from diseased dogs, cats, and horses, only five isolates (from dogs) (17%) carried *bla*<sub>CTX-M-15</sub>. In addition, a lower prevalence of *bla*<sub>CTX-M-15</sub> was found in Switzerland, where eight of the 107 *E. coli* isolates obtained from canine urine samples (7.4%) were ESBLs and all carried *bla*<sub>CTX-M-15</sub>.<sup>4</sup> Furthermore, only one *E. coli* isolate carried this gene in a similar study in Italy and no *bla*<sub>CTX-M</sub> was identified in a study characterizing multidrug-resistant canine urinary *E. coli* isolates from Scotland.<sup>38,39</sup>

*E. coli* carrying *bla*<sub>CTX-M-15</sub> is the most common ESBL type associated with infections in humans in the United Kingdom and Europe.<sup>6</sup> On this basis, the high prevalence of veterinary clinical isolates carrying *bla*<sub>CTX-M-15</sub> identified in this study is worrying both in the context of likely interspecies transfer (man to animals), as well as previous studies identifying animals as a potential reservoir of ESBL-producing *E. coli* for human infection.<sup>40,41</sup> In addition, the clinical and environmental isolates investigated for hospital



**FIG. 1.** Dendrogram showing cluster analysis of *Xba*I PFGE patterns of CTX-M-producing clinical and environmental (EBM) *Escherichia coli* isolates. The columns to the right of the PFGE pattern indicate the ID, ST, PG, and identified beta-lactamases and PMQR genes. PFGE pulsotypes (PT 1–9) were identified as isolates with  $\geq 90\%$  similarity (represented as a vertical dotted line). ID, isolate identification; ST, sequence type; PG, phylogenetic group; PFGE, pulsed-field gel electrophoresis; PMQR, plasmid-mediated quinolone resistance.

dissemination had a common ESBL/AmpC phenotype and genotype and MLST showed that they all belonged to ST410. PCR analysis also demonstrated that all isolates had an identical genetic environment of *bla*<sub>CTX-M-15</sub>, where an IS26 element was inserted in between *bla*<sub>CTX-M-15</sub> gene and its promoter found in *ISEcp1*. PFGE showed 100% homology for the two ESBL/AmpC *E. coli* clinical isolates from the dog with septic peritonitis and the environmental isolates obtained from hospital areas with which this patient came in direct contact (ultrasound table and kennel), or were likely to have spread through staff contact (door handle, the ward fridge handle, and the ward computer keyboard).

This study demonstrated veterinary hospital dissemination of clinical *E. coli* ST410 isolates co-harboring *bla*<sub>CTX-M-15</sub>, *bla*<sub>TEM-1</sub>, *OXA-1* or *CMY-2*, and *acc(6')-Ib-cr*, a genotype conferring MDR and often associated with human clinical isolates.<sup>42–44</sup> Following the confirmation of the ESBL/AmpC phenotype in these isolates, the laboratory contacted the veterinary hospital's infection control team, which took action by cleaning and disinfection of the areas/surfaces identified as sources of these organisms and reinforced hand hygiene policy. The environmental sampling was repeated after reinforcing cleaning and disinfection protocols and no *E. coli* isolates with an ESBL/AmpC phenotype were identified in the subsequent bacterial environmental surveillance specimens. This study demonstrates the role that the microbiology laboratory can play

in the early detection and prevention of MDR isolate dissemination in veterinary hospitals. The presence of multiple  $\beta$ -lactamases in Gram-negative bacteria may interfere with the ESBL phenotypic confirmatory tests<sup>45,46</sup> and it is therefore important that veterinary diagnostic microbiology laboratories are continuously updating their detection methods to recognize ESBL, AmpC, or other emerging resistance phenotypes and to translate the therapeutic or epidemiological significance of these findings to veterinary clinicians. This study also highlights the importance of infection control programs and the benefits of environmental surveillance in the veterinary hospitals for limiting the spread of nosocomial pathogens. Furthermore, the dissemination of the ESBL/AmpC *E. coli* ST410 isolates from veterinary patients (probably from surgical wounds) to the environment, as shown in this study, may indicate a pattern of spread that can occur in the community, especially in the owners home, highlighting the associated human health risk. Therefore, accurate laboratory detection of ESBL/AmpC phenotypes can support the veterinary hospitals in the process of implementing policies for owner's information and infection control advice for limiting the owner's exposure and associated transmission risks.

Recent EUCAST and CLSI guidelines recommend that when using the new interpretative breakpoints, routine ESBL testing is no longer necessary and reporting of susceptibility results to penicillins and cephalosporins for ESBL-producing

Enterobacteriaceae should be 'as found'.<sup>47,48</sup> However, these new guidelines indicate that ESBL screening may still be useful for epidemiological reasons.<sup>47,48</sup> Our findings, demonstrating a high prevalence of CTX-M-15 ESBL-producing *E. coli* in clinical specimens from companion animals, as well as the dissemination of *E. coli* ST410 through the hospital environment, support the need for veterinary laboratories to continue ESBL screening and to continuously upgrade their expertise in detection of complex antimicrobial resistance phenotypes, to benefit both human and animal health.

### Disclosure Statement

All authors declare that they have no conflicts of interest or any other competing financial interests to disclose.

### References

- Thompson, M.F., A.L. Litster, J.L. Platell, and D.J. Trott. 2011. Canine bacterial urinary tract infections: new developments in old pathogens. *Vet. J.* 190:22–27.
- Guardabassi, L., S. Schwarz, and D.H. Lloyd. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother.* 54:321–332.
- Dierikx, C.M., E. van Duijkeren, A.H.W. Schoormans, A. van Essen-Zandbergen, K. Veldman, A. Kant, X.W. Huijsdens, K. van der Zwaluw, J.A. Wagenaar, and D.J. Mevius. 2012. Occurrence and characteristics of extended-spectrum—lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *J. Antimicrob. Chemother.* 67:1368–1374.
- Huber, H., C. Zweifel, M.M. Wittenbrink, and R. Stephan. 2013. ESBL-producing uropathogenic *Escherichia coli* isolated from dogs and cats in Switzerland. *Vet. Microbiol.* 162:992–996.
- Carattoli, A., S. Lovari, A. Franco, G. Cordaro, P. Di Matteo, and A. Battisti. 2005. Extended-spectrum beta-lactamases in *Escherichia coli* isolated from dogs and cats in Rome, Italy, from 2001 to 2003. *Antimicrob. Agents Chemother.* 49:833–835.
- Woodford, N. 2008. Successful, multiresistant bacterial clones. *J. Antimicrob. Chemother.* 61:233–234.
- Ewers, C., M. Grobbel, A. Bethe, L.H. Wieler, and S. Guenther. 2011. Extended-spectrum beta-lactamases-producing Gram-negative bacteria in companion animals: action is clearly warranted! *Berl. Munch. Tierarztl. Wochenschr.* 124:94–101.
- Shaheen, B.W., R. Nayak, S.L. Foley, O. Kweon, J. Deck, M. Park, F. Rafii, and D.M. Boothe. 2011. Molecular characterization of resistance to extended-spectrum cephalosporins in clinical *Escherichia coli* isolates from companion animals in the United States. *Antimicrob. Agents Chemother.* 55:5666–5675.
- Tamang, M.D., H.-M. Nam, G.-C. Jang, S.-R. Kim, M.H. Chae, S.-C. Jung, J.-W. Byun, Y.H. Park, and S.-K. Lim. 2012. Molecular characterization of extended-spectrum-beta-lactamase-producing and plasmid-mediated AmpC beta-lactamase-producing *Escherichia coli* isolated from stray dogs in South Korea. *Antimicrob. Agents Chemother.* 56:2705–2712.
- Dahmen, S., M. Haenni, P. Chatre, and J.-Y. Madec. 2013. Characterization of bla(CTX-M) IncFII plasmids and clones of *Escherichia coli* from pets in France. *J. Antimicrob. Chemother.* 68:2797–2801.
- Shaheen, B.W., R. Nayak, S.L. Foley, and D.M. Boothe. 2013. Chromosomal and plasmid-mediated fluoroquinolone resistance mechanisms among broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates recovered from companion animals in the USA. *J. Antimicrob. Chemother.* 68:1019–1024.
- Hilty, M., B.Y. Betsch, K. Boegli-Stuber, N. Heiniger, M. Stadler, M. Kueffer, A. Kronenberg, C. Rohrer, S. Aebi, A. Endimiani, S. Droz, and K. Muehleemann. 2012. Transmission dynamics of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the Tertiary Care Hospital and the Household Setting. *Clin. Infect. Dis.* 55:967–975.
- Boerlin, P., S. Eugster, F. Gaschen, R. Straub, and P. Schawalder. 2001. Transmission of opportunistic pathogens in a veterinary teaching hospital. *Vet. Microbiol.* 82:347–359.
- Sidjabat, H.E., K.M. Townsend, M. Lorentzen, K.S. Gobius, N. Fegan, J.J.C. Chin, K.A. Bettelheim, N.D. Hanson, J.C. Bensink, and D.J. Trott. 2006. Emergence and spread of two distinct clonal groups of multidrug-resistant *Escherichia coli* in a veterinary teaching hospital in Australia. *J. Med. Microbiol.* 55:1125–1134.
- Sanchez, S., M.A.M. Stevenson, C.R. Hudson, M. Maier, T. Buffington, Q. Dam, and J.J. Maurer. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J. Clin. Microbiol.* 40:3586–3595.
- Sidjabat, H.E., N.D. Hanson, E. Smith-Moland, J.M. Bell, J.S. Gibson, L.J. Filippich, and D.J. Trott. 2007. Identification of plasmid-mediated extended-spectrum and AmpC beta-lactamases in *Enterobacter* spp. isolated from dogs. *J. Med. Microbiol.* 56:426–434.
- Umber, J.K., and J.B. Bender. 2009. Pets and antimicrobial resistance. *Vet. Clin. North Am. Small Anim. Pract.* 39:279–292.
- Stolle, I., E. Prenger-Berninghoff, I. Stamm, S. Scheufen, E. Hassdenteufel, S. Guenther, A. Bethe, Y. Pfeifer, and C. Ewers. 2013. Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. *J. Antimicrob. Chemother.* 68:2802–2808.
- Walther, B., A. Luebke-Becker, I. Stamm, H. Gehlen, A.K. Barton, T. Janssen, L.H. Wieler, and S. Guenther. 2014. Suspected nosocomial infections with multi-drug resistant *E. coli*, including extended-spectrum beta-lactamase (ESBL)-producing strains, in an equine clinic. *Berl. Munch. Tierarztl. Wochenschr.* 127:421–427.
- Haenni, M., C. Ponsin, V. Metayer, C. Medaille, and J.-Y. Madec. 2012. Veterinary hospital-acquired infections in pets with a ciprofloxacin-resistant CTX-M-15-producing *Klebsiella pneumoniae* ST15 clone. *J. Antimicrob. Chemother.* 67:770–771.
- Ewers, C., I. Stamm, Y. Pfeifer, L.H. Wieler, P.A. Kopp, K. Schonning, E. Prenger-Berninghoff, S. Scheufen, I. Stolle, S. Guenther, and A. Bethe. 2014. Clonal spread of highly successful ST15-CTX-M-15 *Klebsiella pneumoniae* in companion animals and horses. *J. Antimicrob. Chemother.* 69:2676–2680.
- Endimiani, A., K.M. Hujer, A.M. Hujer, I. Bertschy, A. Rossano, C. Koch, V. Gerber, T. Francey, R.A. Bonomo, and V. Perreten. 2011. *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. *J. Antimicrob. Chemother.* 66:2248–2254.
- Wohlwend, N., A. Endimiani, T. Francey, and V. Perreten. 2015. Third-Generation-Cephalosporin-Resistant *Klebsiella pneumoniae* Isolates from Humans and Companion Animals in Switzerland: spread of a DHA-Producing

- Sequence Type 11 Clone in a Veterinary Setting. *Antimicrob. Agents Chemother.* 59:2949–2955.
24. McDaniels, A.E., E.W. Rice, A.L. Reyes, C.H. Johnson, R.A. Haugland, and G.N. Stelma. 1998. Confirmational identification of *Escherichia coli*, a comparison of genotypic and phenotypic assays for glutamate decarboxylase and beta-D-glucuronidase. *Appl. Environ. Microbiol.* 64:4113–4113.
  25. Andrews, J.M., and R.A. Howe. 2011. BSAC standardized disc susceptibility testing method (version 10). *J. Antimicrob. Chemother.* 66:2726–2757.
  26. M'Zali, F.H., A. Chanawong, K.G. Kerr, D. Birkenhead, and P.M. Hawkey. 2000. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. *J. Antimicrob. Chemother.* 45:881–885.
  27. Dallenne, C., A. Da Costa, D. Decre, C. Favier, and G. Arlet. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J. Antimicrob. Chemother.* 65:490–495.
  28. Perez-Perez, F.J., and N.D. Hanson. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* 40:2153–2162.
  29. Park, C.H., A. Robicsek, G.A. Jacoby, D. Sahm, and D.C. Hooper. 2006. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother.* 50:3953–3955.
  30. Essack, S.Y., L.M.C. Hall, D.G. Pillay, M.L. McFadyen, and D.M. Livermore. 2001. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum beta-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. *Antimicrob. Agents Chemother.* 45:88–95.
  31. Robicsek, A., J. Strahilevitz, D.F. Sahm, G.A. Jacoby, and D.C. Hooper. 2006. qnr Prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob. Agents Chemother.* 50:2872–2874.
  32. Poirel, L., M. Gniadkowski, and P. Nordmann. 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum beta-lactamase CTX-M-15 and of its structurally related beta-lactamase CTX-M-3. *J. Antimicrob. Chemother.* 50:1031–1034.
  33. Woodford, N., M.E. Ward, M.E. Kaufmann, J. Turton, E.J. Fagan, D. James, A.P. Johnson, R. Pike, M. Warner, T. Cheasty, A. Pearson, S. Harry, J.B. Leach, A. Loughrey, J.A. Lowes, R.E. Warren, and D.M. Livermore. 2004. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. *J. Antimicrob. Chemother.* 54:735–743.
  34. Carattoli, A., A. Bertini, L. Villa, V. Falbo, K.L. Hopkins, and E.J. Threlfall. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63:219–228.
  35. Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* 66:4555–4558.
  36. Wirth, T., D. Falush, R.T. Lan, F. Colles, P. Mensa, L.H. Wieler, H. Karch, P.R. Reeves, M.C.J. Maiden, H. Ochman, and M. Achtman. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* 60:1136–1151.
  37. Schmiedel, J., L. Falgenhauer, E. Domann, R. Bauerfeind, E. Prenger-Berninghoff, C. Imirzalioglu, and T. Chakraborty. 2014. Multiresistant extended-spectrum beta-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol.* 14:187.
  38. Nebbia, P., C. Tramuta, R. Odore, D. Nucera, R. Zanatta, and P. Robino. 2014. Genetic and phenotypic characterisation of *Escherichia coli* producing cefotaximase-type extended-spectrum beta-lactamases: first evidence of the ST131 clone in cats with urinary infections in Italy. *J. Feline Med. Surg.* 16:966–971.
  39. Wagner, S., D.L. Gally, and S.A. Argyle. 2014. Multidrug-resistant *Escherichia coli* from canine urinary tract infections tend to have commensal phylotypes, lower prevalence of virulence determinants and ampC-replicons. *Vet. Microbiol.* 169:171–178.
  40. Naseer, U., and A. Sundsfjord. 2011. The CTX-M Conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microb. Drug Resist.* 17:83–97.
  41. Moodley, A., and L. Guardabassi. 2009. Transmission of IncN Plasmids Carrying bla(CTX-M-1) between Commensal *Escherichia coli* in Pigs and Farm Workers. *Antimicrob. Agents Chemother.* 53:1709–1711.
  42. Lavollay, M., K. Mamlouk, T. Frank, A. Akpabie, B. Burghoffer, S. Ben Redjeb, R. Bercion, V. Gautier, and G. Arlet. 2006. Clonal dissemination of a CTX-M-15 beta-lactamase-producing *Escherichia coli* strain in the Paris Area, Tunis, and Bangui. *Antimicrob. Agents Chemother.* 50:2433–2438.
  43. Maciuca, I.E., N.J. Williams, C. Tuchilus, O. Dorneanu, E. Guguianu, C. Carp-Carare, C. Rambu, and D. Timofte. 2015. High prevalence of *Escherichia coli*-producing CTX-M-15 extended-spectrum beta-lactamases in poultry and human clinical isolates in Romania. *Microb. Drug Resist.* 21:651–662.
  44. Coque, T.M., F. Baquero, and R. Canton. 2008. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro. Surveill.* 13:47.
  45. Poulou, A., E. Grivakou, G. Vrioni, V. Koumaki, T. Pittaras, S. Pournaras, and A. Tsakris. 2014. Modified CLSI extended-spectrum beta-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various beta-lactamases. *J. Clin. Microbiol.* 52:1483–1489.
  46. Willems, E., J. Verhaegen, K. Magerman, S. Nys, and R. Cartuyvels. 2013. Towards a phenotypic screening strategy for emerging beta-lactamases in Gram-negative bacilli. *Int. J. Antimicrob. Agents* 41:99–109.
  47. Leclercq, R., R. Canton, D.F.J. Brown, C.G. Giske, P. Heisig, A.P. MacGowan, J.W. Mouton, P. Nordmann, A.C. Rodloff, G.M. Rossolini, C.J. Soussy, M. Steinbakk, T.G. Winstanley, and G. Kahlmeter. 2013. EUCAST expert rules in antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* 19:141–160.
  48. Clinical and Laboratory Standards Institute. 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-first Informational Supplement. 31. CLSI, Wayne, PA.

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