High prevalence of extended-spectrum ß-lactamase producing Enterobacteriaceae among clinical isolates from cats and dogs admitted to a Veterinary Hospital in Switzerland

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Abstract: Objectives: This study aimed to identify and characterize extended-spectrum -lactamase (ESBL) producing Enterobacteriaceae among clinical samples of companion animals. Methods: A total of 346 non-duplicate Enterobacteriaceae isolates were collected between 2012 and 2016 from diseased cats (n = 115) and dogs (n = 231). The presence of blaESBL, PMQR genes, and the azithromycin resistance gene mph(A) was confirmed by PCR and sequencing of bla genes. Isolates were further characterized by antimicrobial resistance profiling, multilocus sequence typing, phylogenetic grouping, identification of mutations in the QRDR of gyrA and parC, and screening for virulence-associated genes. Results: Among the 346 isolates, 72 (20.8%) were confirmed ESBL producers [58 Escherichia coli (E. coli), 11 Klebsiella pneumoniae (K. pneumoniae), and 3 Enterobacter cloacae]. The strains were cultured from urine (n = 45), skin and skin wounds (n = 8), abscesses (n = 6), surgical sites (n = 6), bile (n = 4), and other sites (n = 3). ESBL genes included blaCTX-M-1, 14, 15, 27, 55, and blaSHV-12, predominantly blaCTX-M-15 (54.8%, 40/73), and blaCTX-M-1 (24.7%, 18/73). Further genes included qnrB (4.2%, 3/72), qnrS (9.7%, 7/72), aac(6')-Ib-cr (47.2%, 34/72), and mph(A) (38.9%, 28/72). Seventeen (23.6%) isolates belonged to the major lineages of human pathogenic K. pneumoniae ST11, ST15, and ST147 and E. coli ST131. The most prevalent ST was E. coli ST410 belonging to phylogenetic group C. Conclusion: The high prevalence of ESBL producing clinical Enterobacteriaceae from cats and dogs in Switzerland and the presence of highly virulent human-related K. pneumoniae and E. coli clones raises concern about transmission prevention as well as infection management and prevention in veterinary medicine.

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High Prevalence of Extended-Spectrum ß-Lactamase producing Enterobacteriaceae among Clinical Isolates from Cats and Dogs in Switzerland

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Abstract

Objectives: This study aimed to identify and characterize extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae among clinical samples of companion animals.

Methods: A total of 346 non-duplicate Enterobacteriaceae isolates were collected between 2012 and 2016 from diseased cats (n=115) and dogs (n=231). The presence of $bla_{ESBL,}$ PMQR genes and the azithromycin resistance gene *mph(A)* was confirmed by PCR and sequencing of *bla* genes. Isolates were further characterized by antimicrobial resistance profiling, multilocus sequence typing, phylogenetic grouping, identification of mutations in the QRDR of *gyrA* and *parC* and screening for virulence associated genes.

Results: Among the 346 isolates, 72 (20.8%) were confirmed ESBL producers (58 *E. coli*, 11 *Klebsiella pneumoniae*, and three *Enterobacter cloacae*). The strains were cultured from urine (n=45), skin and skin wounds (n=8), abscesses (n=6), surgical sites (n=6), bile (n=4), and other sites (n=3). ESBL genes included $bla_{CTX\text{-}M\text{-}1, 14, 15, 27, 55}$, and $bla_{SHV\text{-}12}$, predominantly $bla_{CTX\text{-}M\text{-}15}$ (54.8%, 40/73), and $bla_{CTX\text{-}M\text{-}1}$ (24.7%, 18/73). Further genes included *qnrB* (4.2%, 3/72), *qnrS* (9.7%,7/72), *aac(6')-Ib-cr* (47.2%, 34/72), and *mph(A),* (38.9%, 28/72). Seventeen (23.6%) isolates belonged to the major lineages of human pathogenic *K. pneumoniae* ST11, ST15, and ST147 and *E. coli* ST131. The most prevalent ST was *E. coli* ST410 belonging to phylogenetic group C.

Conclusions: The high prevalence of ESBL producing clinical Enterobacteriaceae from cats and dogs in Switzerland and the presence of highly virulent human related *K. pneumoniae* and *E. coli* clones raises concern about transmission prevention as well as infection management and prevention in veterinary medicine.

Introduction

Members of the family of the Enterobacteriaceae, although natural inhabitants of the intestinal tracts of mammals, may cause urinary tract, skin, ear, soft tissue and respiratory infections in cats and dogs (Koenig, 2012). For uncomplicated infections, first-line therapeutic options are ampicillin, amoxicillin-clavulanate or first and second-generation cephalosporins, while amikacin, third-generation cephalosporins, or fluoroquinolones (enrofloxacin or ciprofloxacin) remain appropriate for severe infections (Koenig, 2012; Scott Weese et al., 2011). One of the most important mechanisms of antimicrobial resistance in Enterobacteriaceae is the enzymatic inactivation of penicillins and cephalosporins by means of plasmid-mediated extended-spectrum ß-lactamases (ESBLs) such as the TEM-, SHV- or CTX-M-group enzymes (Bush, 2013). The emergence of ESBL producing Enterobacteriaceae in healthy and in diseased companion animals constitutes an increasing challenge to infection management in veterinary therapy. Moreover, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like aminoglycosides, fluoroquinolones, and sulfamethoxazole/trimethoprim, which are antimicrobials that are critically important in human medicine (Coque et al., 2008; World Health Organization, 2011). Additionally, previous studies have shown that multidrug resistant, highly virulent human related clonal lineages of Enterobacteriaceae such as *Escherichia coli* belonging to sequence type (ST)131 and ST648, or *Klebsiella pneumoniae* ST11, ST15, and ST147 may be isolated from companion animals (Bogaerts et al., 2015; Ewers et al., 2014a). Consequently, there is growing concern that ESBL producers in companion animals pose a potential health hazard to humans, either through direct transmission of resistant pathogens from animals to humans, or indirectly through transmission of resistance genes (Pomba et al., 2017; Ewers et al., 2014b). Recent data on the prevalence of ESBL producers in clinical isolates of cats and dogs and the phenotypes and genotypes of such isolates are scarce for Switzerland and it remains unclear to what extent clinically relevant phylogenetic or clonal lineages occur.

Here, we analyze a collection of clinical feline and canine Enterobacteriaceae obtained during 2012-2016 by i) identifying ESBL producers within the strain collection, ii) assessing their antimicrobial resistance profiles, iii) determining their bla_{ESBL} genes and screening for plasmid-mediated fluoroquinolone and azithromycin resistance genes and by (iii), characterizing *E. coli* and *K. pneumoniae* strains by multilocus sequence typing (MLST), and *E. coli* strains by phylogenetic grouping and virulence gene profiling.

Material and Methods

Bacterial isolates

Between 2012 and 2016, 346 clinical Enterobacteriaceae were isolated from cats (n=115) and dogs (n=231) at the veterinary clinic of the University of Zürich. The isolates were cultured from urinary samples (n=273), samples obtained from surgical sites (n=26), abscess samples (n=16), skin and skin wound samples (n=14), bile samples (n=7), and from samples from other sites (n=10). Strain identification and routine antimicrobial susceptibility profiling was performed using the VITEK® 2 Compact system with AST GN38 cards (Biomérieux, Nürtingen, Germany**)** according to the manufacturer's instructions. The identity of *Enterobacter cloaca*e was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF–MS, Bruker Daltronics, Bremen, Germany). ESBL producers were screened for using the chromogenic medium Brilliance™ ESBL Agar (Oxoid, Hampshire, UK), according to the manufacturer's recommendations. All non-

duplicate isolates growing on ESBL agar were further analyzed. In accordance with local legislation, ethics approval was not required for this study.

Identification of *bla*$_{ESBL}$ genes and antibiotic susceptibility testing

The presence of *bla*$_{ESBL}$ genes was established by PCR and amplicons were sequenced as described previously using primers listed in Supplementary Table 1 (Geser et al., 2012; Woodford et al., 2006; Zurfluh et al., 2015). For the detection of the CTX-M-25 enzyme group, the newly designed primers Gr. 25 CTX-M fw CCTGTGTTTCGCTGCTGTTGG and Gr. 25 CTX-M rv GGCTCTCTGCCTTCGGCTCC, were used.

Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) performance standards, (CLSI., 2016) using the disk-diffusion method and the antibiotics ampicillin (AM), amoxicillin with clavulanic acid (AMC), azithromycin (AZM), cefazolin (CZ), cefepime (FEP), cefotaxime (CTX), chloramphenicol (C), ciprofloxacin (CIP), fosfomycin (FOS), gentamicin (G), kanamycin (K), nalidixic acid (NA), nitrofurantoin (F/M), streptomycin (S), sulfamethoxazole/trimethoprim (SXT), and tetracycline (TE) (Becton Dickinson, Allschwil, Switzerland). Results were interpreted according to CLSI standards. (CLSI., 2016) For azithromycin, an inhibition zone of ≤ 12 mm was interpreted as resistant. Isolates displaying resistance to three or more classes of antimicrobials (counting ß-lactams as one class) were defined as multidrug-resistant (MDR).

Identification of additional antimicrobial resistance genes

The plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qepA*, and the plasmid-mediated azithromycin resistance gene *mph(A)* were detected by PCR as described elsewhere using primers listed in Supplementary Table 1 (Zurfluh et al., 2014a; Ojo et al., 2004).

Quinolone resistant *E. coli* strains were examined for mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, using PCR amplification and sequencing primers as described previously using primers listed in Supplementary Table 1 (Zurfluh et al., 2014a).

Synthesis of primers and DNA custom sequencing was carried out by Microsynth (Balgach, Switzerland) and nucleotide sequences were analyzed with CLC Main Workbench 6.6.1. For database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was used.

Phylogenetic characterization and multilocus sequence typing

Phylogenetic classification of the *E. coli* isolates into one of the eight groups A, B1, B2, C, D, E, F, (*E. coli sensu stricto)*, or *Escherichia* clade I, was performed as described by Clermont *et al.* (Clermont et al., 2013).

ST determination of the *E. coli* isolates was carried out as described by Wirth *et al.* (Wirth et al., 2006). Sequences were imported into the *E. coli* multilocus sequence type (MLST) database website (http://enterobase.warwick.ac.uk) to determine MLST types. Alleles and STs that had not been previously described were termed new ST, but not assigned new numerical designations by the database.

ST determination of the *K. pneumoniae* isolates was performed according to previously described methods (Diancourt et al., 2005). Sequence types were determined according to the *Klebsiella* MLST database (http://bigsdb.pasteur.fr/klebsiella/).

Virulence factor (VF) determination in uropathogenic *E. coli* isolates

E. coli isolates from urinary samples were tested by conventional PCR for the presence of virulence associated genes that mediate adhesion (p-fimbrial adhesion genes *papAH* and

papEF, and the chaperone-usher fimbria *yfcv*), toxins (α-hemolysin *hlyA*), siderophores (the ferric yersiniabactin uptake protein *fyuA*), serum resistance (*traT*), and the right-hand terminus of pathogenicity island (PAI) from *E. coli* strain CFT073, using primers listed in Supplementary Table 1 and conditions described previously (Johnson and Stell, 2000; Spurbeck et al., 2012). The aggregate VF score was defined as the number of unique VF detected for each isolate, counting the PAI marker as one.

Results

During 2012–2016, 20.8% (72/346) of clinical Enterobacteriaceae isolated from cats and dogs were ESBL producers. The isolates originated from seven cats and 65 dogs, amounting to 6% (7/115) of the feline and 28.1% (65/231) of the canine isolates, respectively. The prevalence of ESBL producers was remarkably higher among isolates from dogs than from cats. Overall, ESBL producers (58 *Escherichia coli*, 11 *Klebsiella pneumoniae*, and three *Enterobacter cloacae*) were cultured from 16.5% (45/273) of the urinary samples, 57.1% (8/14) of the skin and skin wound samples, 37.5% (6/16) abscess samples, 23% (6/26) of the samples obtained from surgical sites, 57.1% (4/7) bile samples, and 30% (3/10) of the samples from other sites (Table 1). Among the *E. coli* from urinary samples, 17% (35/205) were ESBL producers (Table 1).

In addition to their resistance to penicillins and extended-spectrum cephalosporins, the isolates were frequently resistant to quinolones and fluoroquinolones, with 88.9% (64/72) resistant to nalidixic acid and 83.3% (60/72) resistant to ciprofloxacin. They were also resistant to sulfamethoxazole/trimethoprim (76.4%, 55/72), to tetracycline (72.2%, 52/72), to the aminoglycosides streptomycin (45.8%, 33/72), gentamycin (37.5%, 27/72), kanamycin (19.4%, 14/72), to chloramphenicol (25%, 18/72), as well as to azithromycin (22.2%, 16/72) and to nitrofurantoin (12.5%, 9/72). One *K. pneumoniae* isolate (1.4%) was resistant to fosfomycin. Overall, 73.6% (53/72) were MDR, and none was pansusceptible (Supplementary Table 2).

In total, 73 ESBL genes were detected among the 72 isolates, including in one *K. pneumoniae* isolate co-harboring $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$ (Table 2). Among the ESBL genes, $bla_{\text{CTX-M-15}}$ predominated (54.8%, 40/73), followed by $bla_{\text{CTX-M-1}}$ (24.7%, 18/73). Other ESBL genes included $bla_{\text{CTX-M-55}}$ (6.8%, 5/73), $bla_{\text{CTX-M-14}}$ and $bla_{\text{SHV-12}}$ (each 5.5%, 4/73) and $bla_{\text{CTX-M-27}}$ (2.7%, 2/73).

In addition to bla_{ESBLs}, other plasmid-mediated resistance genes detected among the 72 isolates included *aac(6')-I*b-cr (47.2%, 34/72), *mph(A)* (38.9%, 28/72), *qnrS* (9.7%, 7/72), *qnrA* and *qnrB* (each 4.2%, 3/72) (Table 2).

The majority of the aac(6')-Ib-cr genes (88.2%, 30/34), the *mph(A)* genes (62%, 18/29), the *qnrB* (66.7%, 2/3) and *qnrS* genes (85.7%, 6/7) was detected in isolates harboring $bla_{\text{CTX-M-15}}$. All *qnrA* were detected together with $bla_{\text{SHV-12}}$ in *E. cloacae* (Table 2).

Phylogenetic analysis of the 58 *E. coli* isolates revealed a predominance of group C (32.8%, 19/58), followed by group A (31%, 18/58), group B2 and group F (each 12%, 7/58), group B1 (8.6%, 5/58), and group D (3.4%, 2/58) (Supplementary Table 2).

Among the 58 *E. coli* isolates, 23 different STs and three new STs were identified (Table 2 and Supplementary Table 3). Most frequently, isolates belonged to ST410 (27.6%, 16/58), followed by a collective of STs occurring only once or twice (24.1%, 14/58), ST361 (13.8%, 8/58), ST131 (12%, 7/58) and ST648, ST744, and new STs (each 5.2%, 3/58). *E. coli* ST410 and human related pandemic clone *E. coli* ST131 were detected only in isolates from dogs. *E. coli* ST410 was isolated from 33.3% of the urine samples from dogs.

Among the 11 *K. pneumoniae* isolates, 4 different STs were detected (Table 2 and Supplementary Table 2). The majority (54.5%, 6/11) of the isolates belonged to ST147. Other STs included ST15 (27.3%, 3/11), ST11, and ST788 (both 9.1%, 1/11).
Among the 51 *E. coli* isolates displaying quinolone resistance, all revealed chromosomal mutations that result in amino acid substitutions in GyrA and ParC. Unusual point mutations Asp87→Tyr in GyrA and Glu84→Gly in ParC were noted for two *E. coli* ST457 isolates harboring $bla_{\text{CTX-M-55}}$ (Table 3 and Supplementary Table 2).
VFs were distributed unequally among the 35 uropathogenic *E. coli* isolates (Table 4). For 42.9% (15/35) of *E. coli* urinary isolates, no VF were detected. Strains with aggregate VF score ≥1 were identified in 34.5% (57.1%/35) of the isolates. VF scores were highest for isolates belonging to ST617 (median 5.5, range 5-6) and ST131 (median 7, range 4-7).

Discussion

This study identified a high prevalence (20.8%) of ESBL producing Enterobacteriaceae derived from clinical samples of cats and dogs collected during 2012-2016 in Switzerland, which is considerably higher than that found in similar studies from pets in the UK (7%) (Timofte et al., 2016), the Netherlands (2%) (Dierikx et al., 2012), and France (3.7%) (Dahmen et al., 2013), and remarkably higher than the prevalence of 1.6% detected in a European collection of Enterobacteriaceae obtained from diseased companion animals in 2015 (Bogaerts et al., 2015). In addition, among the uropathogenic *E. coli* analyzed in this study, the observed prevalence of 16.8% of ESBL producers is considerably higher than that found previously in cats and dogs in Switzerland between 2010–2012 (7.5%) (Huber et al., 2013).
Overall, a diversity of bla_{ESBL} genes was found within three bacterial species. The predominance of $bla_{\text{CTX-M-15}}$, which is highly prevalent in ESBL producers in humans, is comparable to what is found in other studies on isolates from companion animals (Dahmen et al., 2013; Shaheen et al., 2011; Timofte et al., 2016). This gene was the only one that was detected in cats and dogs in Switzerland between 2010–2012 (Huber et al., 2013). Our study shows that in the following years, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-12}}$ harboring Enterobacteriaceae have emerged in cats and dogs in Switzerland.
Second to $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-1}}$ was the most frequent variant identified in this study. The $bla_{\text{CTX-M-1}}$ gene is the most prevalent bla_{ESBL} gene among ESBL producing Enterobacteriaceae isolated food producing animals and food, in particular chicken and chicken meat (Zurfluh et al., 2014b; Abgottspon et al., 2014). Consumption of raw meat represents a risk factor for dogs acquiring pathogenic *E. coli,* including ESBL producers (Glaser et al., 2012; Weese et al., 2005). Moreover, a recent study detected a high prevalence (77.8%) of ESBL producers in raw cat food and demonstrated a strong association of consumption of raw cat food with shedding of ESBL producers by household cats in the Netherlands (Baede et al., 2017). Further studies are needed to investigate the possibility of raw meat as an origin of the high prevalence of ESBL and the occurrence of CTX-M-1 producers in isolates from companion animals in Switzerland. Similarly, CTX-M-55 has been widely reported in food-producing animals and pets in mainland China (Rao et al., 2014). This ESBL variant has rarely been detected outside China and its emergence in pets in Switzerland, possibly due to international food and animal trade, warrants attention.
This study identified 17 (23.6%) isolates belonging to major lineages of human pathogenic *K. pneumoniae* and *E. coli.* CTX-M-15 producing *K. pneumoniae* ST11, ST 15, and ST147 represent major international high-risk nosocomial clones (Woodford et al., 2011). *K. pneumoniae* ST11 and ST15 from companion animals have been involved in nosocomial events in veterinary clinics (Wohlwend et al., 2015; Ewers et al., 2014a). By contrast, *K.*

pneumoniae ST147 has only very recently been detected in pets in Europe and in Japan (Ovejero et al., 2017; Sato et al., 2017), and this is to our knowledge the second report on this sequence type isolated from dogs in Europe.

Pandemic human pathogenic *E. coli* ST131 producing CTX-M-15 has disseminated globally in hospital and community settings causing a wide spectrum of infections including urinary tract infection, cystitis, pyelonephritis, and bacteremia, with transmission between humans and their companion animals (cats and dogs in particular) well documented (Nicolas-Chanoine et al., 2014). Since the earlier study period 2010-2012 (Huber et al., 2013), the prevalence of ESBL producing uropathogenic *E. coli* ST131among feline and canine samples in Switzerland has increased from 0% to 1.5% (4/273), and includes *E. coli* ST131-CTX-M-15 as well as ST131-CTX-M-27, which is currently emerging in human medicine in Germany, France and Japan (Ghosh et al., 2017; Birgy et al., 2017).

Other human related strains detected in this study included *E. cloacae* harboring $bla_{\text{SHV-12}}$ together with the plasmid-mediated quinolone resistance gene *qnrA*. The combined presence of $bla_{\text{SHV-12}}$ and *qnrA* has been described in human clinical *E. cloacae* isolates in hospitals in France and the UK (Cambau et al., 2006; Strahilevitz et al., 2009). Although data on ESBL producing *E. cloacae* in animals are scarce (Dierikx et al., 2012; Haenni et al., 2016), our results provide evidence that this important pathogen has emerged in companion animals in Switzerland, illustrating their potential for increased dissemination.

In this study, the identification of phylogenetic groups among the *E. coli* isolates was performed based on the new Clermont scheme (Clermont et al., 2013). Consequently, a number of STs from this study were classified as phylogenetic group F from their original D designation, including *E. coli* ST117 which is a recognized avian pathogenic lineage (Mora et al., 2012), *E. coli* ST354 and ST648, which are frequently detected in humans and animals (Vangchhia et al., 2016; Ewers et al., 2014b), and the rarely described *E. coli* ST457. In this study, we detected two isolates belonging to ST457, both harboring the uncommon $bla_{\text{CTX-M-55}}$. *E. coli* ST457-CTX-M-55 harboring the carbapenemase gene $bla_{\text{KPC-3}}$ was isolated in Italy from a human diagnosed with pneumonia (Accogli et al., 2014), but to our knowledge, this ST has not been associated with disease in companion animals before.

A large number (26.4%, 19/72) of isolates changed designation from the original phylogenetic group A to group C. Most isolates in this group belonged to ST410 and were of low virulence. However, the panel of VFs selected for this study was limited in number and represents only a subset of known VFs. Other important determinants of virulence may have been missed due to this limitation. Nevertheless, the pathogenic potential of ST410 has been documented previously, together with strong evidence for clonal dissemination of *E. coli* ST410 between the avian wildlife, humans, and companion animals in Germany (Schaufler et al., 2016; Falgenhauer et al., 2016). CTX-M-15 producing *E. coli* ST410 was also identified as a veterinary hospital strain in the UK (Timofte et al., 2016). Although currently available reports on bla_{ESBLs} in ST410 are limited to $bla_{\text{CTX-M-15}}$, our results demonstrate that this ST can also harbor $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-55}}$, both variants that occur among food producing animals (Zurfluh et al., 2014b; Rao et al., 2014). Here, we provide further evidence for the pathogenic potential of this ST in companion animals and suggest that, in addition to its potential as an international clone for the dissemination of $bla_{\text{CTX-M-15}}$, it may contribute to the dispersion of other resistance genes including other *bla*ESBL variants, *aac(6')-Ib-cr* and *mph(A)*. The high prevalence (38.9%) of isolates harboring plasmid-mediated *mph(A)* which confers reduced susceptibility to azithromycin is of concern, since this macrolide is considered a last-resort antimicrobial agent for shigellosis (Baker et al., 2015). Furthermore, azithromycin represents an option for the treatment of gram-negative rods expressing MDR, including carbapenem-resistant isolates of *Pseudomonas aeruginosa*, *K. pneumoniae*, and

Acinetobacter baumannii (Lin et al., 2015), and is the only antimicrobial under consideration for the treatment of enterohemorrhagic *E. coli* in humans (Jost et al., 2016).

In conclusion, this study provides information on the prevalence, the bla_{ESBL} variants and the genotypes of ESBL producing isolates in cats and dogs in Switzerland. The occurrence of potentially high-risk human related *K. pneumoniae* and *E. coli* clones, as well as *E. cloacae* harboring bla_{SHV-12} and *qnrA* previously described in humans suggests transmission events between companion animals as well as the possibility of the presence of a common source. This collection of ESBL producing Enterobacteriaceae from cats and dogs identifies *E. coli* phylogroup C ST410 as a frequent MDR, ESBL producing clone among clinical isolates from dogs in Switzerland that warrants further attention. The clinical significance of phylogroup C strains as etiological agents of extraintestinal disease and disseminators of antimicrobial resistance in companion animals remains to be investigated. Understanding the epidemiological and molecular features of ESBL-producing Enterobacteriaceae in companion animals can be helpful for infection management and prevention in veterinary as well as in human medicine.

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Conflict of Interest Statement

None to declare.

Author Contributions Statement

RS, designed the study, AZ, KZ, SNS and SS carried out the microbiological and molecular biological tests. AZ, KZ, SNS, and MNI analyzed and interpreted the data. MNI drafted the manuscript. All authors read and approved the final manuscript.

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512

513 Table 1: Percent and distribution of ESBL producers among clinical Enterobacteriaceae from cats and dogs in Switzerland 2012–2016.
514

Host	Source	*Escherichia coli*			*Klebsiella pneumoniae*			*Enterobacter cloacae*			Other species[a]			Total Enterobacteriaceae		
		No.	No. ESBL	% ESBL	No.	No. ESBL	% ESBL	No.	No. ESBL	% ESBL	No.	No. ESBL	% ESBL	No.	No. ESBL	% ESBL
Cats	urine	74	1	1.4	6	2	33.3	10	0	0	6	0	0	96	3	3.1
Cats	surgical sites	4	1	25	0	0	0	2	0	0	0	0	0	6	1	16.6
Cats	abscess	3	1	33.3	0	0	0	2	0	0	0	0	0	5	1	20
Cats	wound/skin	3	1	33.3	0	0	0	0	0	0	0	0	0	3	1	33.3
Cats	bile	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Cats	other	2	1	50	0	0	0	0	0	0	0	0	0	2	1	50
Dogs	urine	131	34	26	25	5	20	10	3	30	11	0	0	177	42	23.7
Dogs	surgical sites	13	5	38.5	3	0	0	4	0	0	0	0	0	20	5	25
Dogs	abscess	6	4	66.7	3	1	33.3	0	0	0	2	0	0	11	5	45.5
Dogs	wound/skin	6	5	83.3	2	2	100	1	0	0	2	0	0	11	7	63.6
Dogs	bile	4	4	100	0	0	0	0	0	0	0	0	0	4	4	100
Dogs	other	5	1	20	1	1	100	2	0	0	0	0	0	8	2	25

515 [a] Other species included *Citrobacter freundii*, *Citrobacter koseri*, *Proteus mirabilis, Proteus vulgaris*
516

517 Table 2: Type and distribution of ESBL genes and other plasmid-mediated resistance genes among 72 clinical Enterobacteriaceae isolated from
518 cats and dogs in Switzerland 2012-2016.
519

Host	Species	No. isolates	Source (n)	MLST (n)	*bla*$_{ESBL}$	Additional plasmid-mediated AMR determinants
Cat	*E. coli*	1	urine (1)	10 (1)	*bla*$_{CTX-M-1}$	-
Cat	*E. coli*	1	other (1)	23 (1)	*bla*$_{CTX-M-1}$	*mph(A)*, *qnrS*
Cats	*E. coli*	3	abscess (1), wound (1), surgical site (1)	361 (2), 648 (1)	*bla*$_{CTX-M-15}$	*mph(A)*, *aac(6')-Ib-cr*
Cat	*K. pneumoniae*	1	urine (1)	15 (1)	*bla*$_{CTX-M-15}$	*aac(6')-Ib-cr*
Cat	*K. pneumoniae*	1	urine (1)	147 (1)	*bla*$_{CTX-M-15}$	*qnrS*
Dogs	*E. coli*	13	urine (9), abscess (1), wound (1), surgical site (2)	58 (1),101 (2), 117 (1), 410 (5), 617 (2), 1431 (1), new ST (1)	*bla*$_{CTX-M-1}$	-
Dog	*E. coli*	1	Surgical site (1)	3889 (1)	*bla*$_{CTX-M-1}$	*mph(A)*,
Dog	*E. coli*	1	bile (1)	90 (1)	*bla*$_{CTX-M-1}$	*mph(A)*, *aac(6')-Ib-cr*
Dog	*K. pneumoniae*	1	urine (1)	788 (1)	*bla*$_{CTX-M-1}$	*mph(A)*, *qnrB*
Dogs	*E. coli*	2	urine (2)	744 (2)	*bla*$_{CTX-M-14}$	-
Dogs	*E. coli*	2	urine (1), wound (1)	744 (1), 131 (1)	*bla*$_{CTX-M-14}$	*mph(A)*
Dogs	*E. coli*	3	urine (3)	131 (1), 354 (1), 648 (1)	*bla*$_{CTX-M-15}$	-
Dog	*E. coli*	1	urine (1)	533 (1)	*bla*$_{CTX-M-15}$	*mph(A)*
Dogs	*E. coli*	10	urine (7), bile (1), wound (1), surgical site (1)	131 (3), 410 (7)	*bla*$_{CTX-M-15}$	*aac(6')-Ib-cr*
Dogs	*E. coli*	12	urine (5), abscess (3), bile (1), wound (1), surgical site (1), other (1)	131 (1), 167 (2), 361 (6), 410 (2), new ST (1)	*bla*$_{CTX-M-15}$	*mph(A)*, *aac(6')-Ib-cr*
Dog	*E. coli*	1	urine (1)	new ST (1)	*bla*$_{CTX-M-15}$	*mph(A), aac(6')-Ib-cr; qnrB*
Dogs	*K. pneumoniae*	4	urine (3), other (1)	147 (4)	*bla*$_{CTX-M-15}$	*qnrS*
Dogs	*K. pneumoniae*	2	wound (2)	15 (2)	*bla*$_{CTX-M-15}$	*aac(6')-Ib-cr*
Dog	*K. pneumoniae*	1	abscess (1)	11 (1)	*bla*$_{CTX-M-15}$	*mph(A), aac(6')-Ib-cr , qnrB*

Table 2 continued

Host	Species	No. isolates	Source (n)	MLST (n)	*bla*$_{ESBL}$	Additional plasmid-mediated AMR determinants
Dog	*K. pneumoniae*	1	urine (1)	147 (1)	*bla*$_{CTX-M-15}$, *bla*$_{SHV-12}$	*qnrS*
Dog	*E. coli*	1	urine (1)	131 (1)	*bla*$_{CTX-M-27}$	*mph(A)*,
Dog	*E. coli*	1	bile (1)	648 (1)	*bla*$_{CTX-M-27}$	*mph(A), aac(6')-Ib-cr*
Dogs	*E. coli*	3	urine (2), wound (1)	457 (2), 1177 (1)	*bla*$_{CTX-M-55}$	-
Dogs	*E. coli*	2	urine (2)	410 (2)	*bla*$_{CTX-M-55}$	*mph(A), aac(6')-Ib-cr*
Dogs	*E. cloacae*	3	urine (3)	–	*bla*$_{SHV-12}$	*qnrA*

aac(6')-Ib-cr, aminoglycoside 6'-N-acetyltransferase variant; AMR, antimicrobial resistance; *bla*, ß-lactamase gene; MLST, multilocus sequence type; *mph(A)*, macrolide 2'-phosphotransferase gene; *qnr*, quinolone resistance gene; –, not determined; -, not present.

Table 3: Amino acid substitutions in the QRDR of 51 quinolone resistant ESBL producing *E. coli* from cats and dogs, Switzerland 2012-2016.

	QRDR					
	gyrA			*parC*		
Host (n=51)	Ser83→Leu n (%)	Asp87→Asn n (%)	Asp87→Tyr n (%)	Ser80→Ile n (%)	Glu84→Val n (%)	Glu84→Gly n (%)
Cats (n=4)	4 (100)	3 (75)	0 (0)	0 (75)	0 (0)	0 0)
Dogs (n=47)	47 (100)	44 (93.6)	2 (4.3)	46 (97.9)	7 (14.9)	1 (2.1)

Asn, asparagine; Asp, aspartic acid; CIP, ciprofloxacin; Glu, glutamic acid; Gly, glycine; *gyrA*, DNA gyrase (type II topoisomerase) gene; Ile, isoleucine; Leu, leucine; *parC,* topoisomerase IV gene; QRDR, quinolone resistance determining region; Ser, serine; Tyr, tyrosine; Val, valine.

Table 4: Virulence associated genes detected in 35 uropathogenic ESBL producing *E. coli* from cats and dogs in Switzerland 2012-2016.

Host	No. isolates	PG	ST	CC	*papAH*	*papEF*	*yfcv*	*hlyA*	*fyuA*	*traT*	PAI	Plasmid-mediated resistance gene(s)
Dog	1	A	617	10	+	+	-	+	+	+	+	*bla*$_{\text{CTX-M-1}}$
Dog	1	A	617	10	+	+	-	-	+	+	+	*bla*$_{\text{CTX-M-1}}$
Dog	2	A	361	–	-	-	-	-	-	-	-	*aac(6')-Ib-cr, bla*$_{\text{CTX-M-15}}$, *mph(A)*
Dog	1	A	361	–	-	-	-	-	+	+	-	*aac(6')-Ib-cr, bla*$_{\text{CTX-M-15}}$, *mph(A)*
Dog	1	A	744	–	-	-	-	-	-	+	-	*mph(A)*, *bla*$_{\text{CTX-M-14}}$
Dog	2	A	744	–	-	-	-	-	-	-	-	*bla*$_{\text{CTX-M-14}}$
Dog	1	B1	533	–	-	-	-	-	-	+	-	*mph(A)*, *bla*$_{\text{CTX-M-15}}$
Dog	1	B1	1431	–	-	-	-	+	+	+	-	*bla*$_{\text{CTX-M-1}}$
Dog	2	B2	131	131	+	+	+	+	+	+	+	*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-15}}$
Dog	1	B2	131	131	+	+	+	+	+	+	+	*bla*$_{\text{CTX-M 15}}$
Dog	1	B2	131	131	-	-	+	-	+	+	+	*mph(A)*, *bla*$_{\text{CTX-M-27}}$
Cat	1	C	23	23	-	-	-	-	+	-	-	*bla*$_{\text{CTX-M-1}}$
Dog	5	C	410	23	-	-	-	-	-	-	-	*bla*$_{\text{CTX-M-1}}$
Dog	5	C	410	23	-	-	-	-	-	-	-	*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-15}}$,
Dog	1	C	410	23	+	-	-	-	+	-		*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-15}}$, *mph(A)*
Dog	1	C	410	23	-	-	-	-	-	-	-	*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-15}}$, *mph(A)*
Dog	2	C	410	23	-	-	-	+	-	-	-	*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-55}}$, *mph(A)*
Dog	1	C	nd	nd	-	-	-	+	-	-	-	*aac(6')-Ib-cr*, *bla*$_{\text{CTX-M-15}}$, *mph(A), qnrB*
Dog	1	D	1177	–	-	-	-	-	+	+	-	*bla*$_{\text{CTX-M-55}}$
Dog	1	F	117	–	-	-		-	-	+	-	*bla*$_{\text{CTX-M-1}}$
Dog	1	F	354	354	-	-	+	-	-	+	-	*bla*$_{\text{CTX-M-15}}$
Dog	1	F	457	–	-	-	+	-	-	+	+	*bla*$_{\text{CTX-M-55}}$
Dog	1	F	648	648	-	-	+	-	+	+	+	*bla*$_{\text{CTX-M-15}}$

aac(6')-Ib-cr, aminoglycoside 6'-N-acetyltransferase variant; *bla*, ß-lactamase gene; CC, clonal complex; *fyuA*, ferric yersiniabactin uptake protein gene; *hlyA*, α-hemolysin gene; *mph(A)*, macrolide 2'-phosphotransferase gene; nd, not determined; PAI, right-hand terminus of pathogenicity island; *papAH* and *papEF*, p-fimbrial adhesion genes; PG, phylogenetic group; *qnrB*, quinolone resistance gene; ST, sequence type; *traT*, serum resistance gene; *yfcv,* chaperone-usher fimbria gene; +, presence of a trait; -, absence of a trait; –, not applicable.