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Antimicrobial resistance, multilocus sequence types and virulence profiles of ESBL producing and non-ESBL producing uropathogenic Escherichia coli isolated from cats and dogs in Switzerland

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Abstract: Unter 64 uropathogenen Escherichia coli (UPEC), welche von 13 Katzen und 51 Hunden isoliert wurden, fanden sich 35 extended-spectrum -Lactamase (ESBL) produzierende UPEC, die restlichen 29 waren nicht-ESBL Bildner. 46 (71.9%) Isolate waren multiresistent (MDR). Innerhalb der ESBL Bildner wurden folgende blaESBL Gene identifiziert: blaCTX-M-15 (n=17/48.6%), blaCTX-M-1 (n=10/28.6%), blaCTX-M-55 (n=4/11.4%), blaCTX-M-14 (n=3/8.6%) und blaCTX-M-27 (n=1/2.9%). Die Plasmid-mediierten Gene der Fluorchinolonresistenz aac(6')-Ib- cr und qnrB sowie das Azithromycinresistenzgen mph(A) wurden in 17 (26.6%) Isolaten, beziehungsweise in einem (1.6%) und in 13 (20.3%) gefunden. Die am häufigsten vorkommenden Phylogruppen waren Gruppe C (n=19) und B2 (n=15). Es wurden 26 verschiedene Sequenz-Typen (ST) identifiziert, wobei zwei davon neu waren. Die häufigsten ST waren ST410 (n=16/25%), ST131 und ST73 (beide n=5/7.8%) und ST361 (n=4/6.3%). Zehn (15.6%) der ST stehen in Verbindung zu Harnwegsinfektionen (HWI) beim Menschen. Von den sieben Virulenz-assoziierten Genen war fyuA das am häufigsten vorkommende. Die Gesamtzahl an Virulenzfaktoren (VF) war am höchsten bei Isolaten, die zur Phylogruppe B2 (mittlere Gesamtzahlrate 6, Hauptrate 5.5, Streubreite 3-7) gehören, am niedrigsten für Isolate der Phylogruppe C (0/ 0.5/ 0-3). Der häufigste ST in dieser Studie, ST410, wies die geringste Gesamtzahl an VF (0/ 0.3/ 0-2) auf. Among 64 uropathogenic Escherichia coli (UPEC) isolated from 13 cats and 51 dogs, 35 were extended-spectrum beta-lactamase (ESBL) producers, and 29 were non-ESBL producers. Forty-six (71.9%) of the isolates were multidrug resistant (MDR). Among the ESBL producers, blaCTX-M-15 (n=17/48.6% of the blaESBLs), blaCTX-M-1 (n=10/28.6%), blaCTX-M- 55 (n=4/11.4%), blaCTX-M-14 (n=3/8.6%), and blaCTX-M-27 (n=1/2.9%) were identified. The plasmid-mediated fluoroquinolone resistance genes aac(6')-Ib-cr, qnrB and the azithromycin resistance gene mph(A) were detected in 17 (26.6% of all isolates), one (1.6%) and in 13 (20.3%) respectively. The most frequent phylogenetic groups were C (n=19) and B2 (n=15). Twenty-six different sequence types (STs) were identified, with two being novel. The most frequent STs were ST410 (n=16/25%), ST131, and ST73 (both n=5/7.8%), and ST361 (n=4/6.3%). Ten (15.6%) of the STs have been associated with urinary tract infection (UTI) in humans, suggesting zoonotic potential. Among seven virulence-associated genes, fyuA was the most prevalent. The overall aggregate virulence factor (VF) score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3- 7), and lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3). The most frequent ST in this study, ST410, harboured the lowest number of VF (0/0,3/0-2).

Other titles: Antibiotikaresistenz, MLST Sequenztypen und Virulenzprofile von ESBL-bildenden und nicht ESLB-bildenden uropathogenen Escherichia coli Isolaten von Katzen und Hunden in der Schweiz

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Contents

Antimicrobial resistance, multilocus sequence types and virulence profiles of ESBL producing and non-ESBL producing uropathogenic *Escherichia coli* isolated from cats and dogs in Switzerland

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In Fulfillment of the Doctoral Thesis of Anna Lena Zogg

Abstract

Among 64 uropathogenic *Escherichia coli* (UPEC) isolated from 13 cats and 51 dogs, 35 were extended-spectrum beta-lactamase (ESBL) producers, and 29 were non-ESBL producers. Forty-six (71.9%) of the isolates were multidrug resistant (MDR). Among the ESBL producers, *bla*$_{CTX-M-15}$ (n=17/48.6% of the *bla*$_{ESBLs}$), *bla*$_{CTX-M-1}$ (n=10/28.6%), *bla*$_{CTX-M-55}$ (n=4/11.4%), *bla*$_{CTX-M-14}$ (n=3/8.6%), and *bla*$_{CTX-M-27}$ (n=1/2.9%) were identified. The plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr*, *qnrB* and the azithromycin resistance gene *mph(A)* were detected in 17 (26.6% of all isolates), one (1.6%) and in 13 (20.3%) respectively. The most frequent phylogenetic groups were C (n=19) and B2 (n=15). Twenty-six different sequence types (STs) were identified, with two being novel. The most frequent STs were ST410 (n=16/25%), ST131, and ST73 (both n=5/7.8%), and ST361 (n=4/6.3%). Ten (15.6%) of the STs have been associated with urinary tract infection (UTI) in humans, suggesting zoonotic potential. Among seven virulence-associated genes, *fyuA* was the most prevalent. The overall aggregate virulence factor (VF) score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3-7), and lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3). The most frequent ST in this study, ST410, harboured the lowest number of VF (0/0,3/0-2).

Zusammenfassung

Unter 64 uropathogenen *Escherichia coli* (UPEC), welche von 13 Katzen und 51 Hunden isoliert wurden, fanden sich 35 extended-spectrum β-Lactamase (ESBL) produzierende UPEC, die restlichen 29 waren nicht-ESBL Bildner. 46 (71.9%) Isolate waren multiresistent (MDR). Innerhalb der ESBL Bildner wurden folgende bla_{ESBL} Gene identifiziert: $bla_{CTX-M-15}$ (n=17/48.6%), $bla_{CTX-M-1}$ (n=10/28.6%), $bla_{CTX-M-55}$ (n=4/11.4%), $bla_{CTX-M-14}$ (n=3/8.6%) und $bla_{CTX-M-27}$ (n=1/2.9%). Die Plasmid-mediierten Gene der Fluorchinolonresistenz *aac(6')-Ib-cr* und *qnrB* sowie das Azithromycinresistenzgen *mph(A)* wurden in 17 (26.6%) Isolaten, beziehungsweise in einem (1.6%) und in 13 (20.3%) gefunden. Die am häufigsten vorkommenden Phylogruppen waren Gruppe C (n=19) und B2 (n=15). Es wurden 26 verschiedene Sequenz-Typen (ST) identifiziert, wobei zwei davon neu waren. Die häufigsten ST waren ST410 (n=16/25%), ST131 und ST73 (beide n=5/7.8%) und ST361 (n=4/6.3%). Zehn (15.6%) der ST stehen in Verbindung zu Harnwegsinfektionen (HWI) beim Menschen. Von den sieben Virulenz-assozierten Genen war *fyuA* das am häufigsten vorkommende. Die Gesamtzahl an Virulenzfaktoren (VF) war am höchsten bei Isolaten, die zur Phylogruppe B2 (mittlere Gesamtzahlrate 6, Hauptrate 5.5, Streubreite 3-7) gehören, am niedrigsten für Isolate der Phylogruppe C (0/ 0.5/ 0-3). Der häufigste ST in dieser Studie, ST410, wies die geringste Gesamtzahl an VF (0/ 0.3/ 0-2) auf.

Introduction

Escherichia coli occurs naturally in the digestive tract of humans and animals as a commensal, however, some strains of *E. coli* can cause extraintestinal diseases, such as pneumonia, sepsis, meningitis and urinary tract infections (UTI) (Kaper et al., 2004).

Commensal strains have been shown by phylogenetic analyses to be associated with phylogenetic group A, B1 or C, while virulent extraintestinal *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D, E, or F (Clermont et al., 2013).

Uropathogenic *E. coli* (UPEC) is the most common etiologic agent of UTI in humans and animals (Foxman, 2010; Olin and Bartges, 2015). The ability of UPEC to cause disease is associated with the expression of specific virulence factors (VF) encoding adhesins, invasins, toxins and other proteins (Kaper et al., 2004).

Among companion animals, *E. coli* clones that are found in the intestine may cause urinary tract infection (UTI) (Johnson et al., 2008b). Furthermore, UPEC may be acquired via external reservoirs: previous studies have demonstrated that humans and their companion animals can share pathogenic types of *E. coli,* including UPEC, indicating zoonotic as well as anthroponotic transmission (Johnson et al., 2008b; Johnson et al., 2008c; Johnson et al., 2009). Other significant sources of pathogenic *E. coli* include raw meat canine diets and raw or inadequately cooked chicken meat (Glaser 2012; Schmidt et al., 2015).

For uncomplicated UTI in cats and dog, the first-line therapeutic option is amoxicillin, with sulfamethoxazole/trimethoprim and fluoroquinolones remaining appropriate for complicated infections and pyelonephritis, respectively (Barsanti, 2012). The emergence of antimicrobial resistance among UPEC is of great concern and increases the risk of antimicrobial treatment failure in companion animals, as in humans.

Understanding the prevalence of antimicrobial resistance and pathogenicity of UPEC isolated from cats and dogs is important both from veterinary medicine and public health perspectives, but information regarding the population structure and virulence association of UPEC from cats and dogs is still limited.

The aim of this study was therefore to (i) assess the antimicrobial susceptibility of uropathogenic *E. coli* isolates obtained throughout 2012–2016 from Swiss dogs and cats, (ii) to detect and characterize plasmid-mediated resistance to third-generation cephalosporins, fluoroquinolones and azithromycin, (iii) to characterize the strains by phylogenetic grouping, multilocus sequence typing (MLST) and virulence profiling.

Materials and methods

Bacterial isolates

From a total of 205 *Escherichia coli* strains isolated between 2012 and 2016 from urine samples of companion animals admitted to the University of Zürich veterinary clinic, a total of 64 (35 ESBL-producing and 29 randomly selected non-ESBL producing) strains from 13 cats and 51 dogs were analysed. Strain identification and preliminary antimicrobial susceptibility profiling was performed using VITEK 2 Compact system with AST GN38 cards (Biomérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Phenotypic and genotypic antimicrobial resistance testing

Antimicrobial susceptibility testing was performed using the disk-diffusion method and the antibiotics ampicillin (AM), cefazolin (CZ), cefotaxime (CTX), amoxicillin with clavulanic acid (AMC), cefepime (FEP), nalidixic acid (NA), ciprofloxacin (CIP), sulfamethoxazole/trimethoprim (SXT), fosfomycin (FOS), nitrofurantoin (F/M), streptomycin (S), kanamycin (K), gentamicin (G), chloramphenicol (C) and tetracycline (TE) (Becton Dickinson, Allschwil, Switzerland). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) performance standards (CLSI, 2016). Isolates displaying resistance to three or more classes of antimicrobials (counting beta-lactams as one class) were defined as multidrug-resistant (MDR). Synergistic effects between AMC and CTX and FEP were regarded as an indication of the presence of an ESBL producer (Kaur et al., 2013).

For genotypic analyses, bacterial DNA was extracted using a standard heat lysis protocol. The presence of bla_{ESBL} genes belonging to the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} families was confirmed by PCR and amplicons were sequenced as described previously (Woodford et al., 2006; Zurfluh et al., 2015). Ciprofloxacin resistant strains were examined for mutations in quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, using previously described PCR and sequencing primers (Zurfluh et al., 2014). Screening all isolates for the plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, and *qepA* was carried out as described previously (Zurfluh et al., 2014). Further, all strains were screened for the plasmid-mediated azithromycin resistance gene *mph(A)* using previously described primers (Ojo et al., 2004). DNA samples from isolates described

previously were used as positive controls (Nüesch-Inderbinen et al., 2017; Zurfluh et al., 2014).

Synthesis of primers and DNA custom sequencing was carried out by Microsynth (Balgach, Switzerland) and nucleotide sequences were analysed 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

The distribution of phylogenetic groups amongst the isolates was determined by the improved phylotyping PCR approach described by Clermont *et al.*, targeting the genes *chuA, yjaA, arpA* and TspE4.C2 (Clermont et al., 2013). Isolates were thereby classified as belonging to one of the eight phylogenetic groups A, B1, B2, C, D, E, F, (*E. coli sensu stricto)*, or *Escherichia* clade I.

For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) were amplified by PCR from DNA, as described by Wirth *et al*. (Wirth et al., 2006). Sequencing of the amplification products was performed by Microsynth (Balgach, Switzerland). Sequences were imported into the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli) to determine sequence types (STs). Alleles and STs that had not been previously described were designated new ST, but not assigned numerical designations, since whole-genome sequencing was not performed.

Virulence factor (VF) determination

All 64 strains were tested by conventional PCR for the presence of genes of VF that mediate adhesion (p-fimbrial adhesion genes *papAH* and *papEF*, and the chaperone-usher fimbria *yfcv*), toxins (α-haemolysin *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 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.

The Based Upon Related Sequence Types (eBURST) clustering algorithm in phyloviz (www.phyloviz.net) was used to visualise relationships between the isolates.

Results

Antimicrobial resistance

Between 2012-2016, 205 *E. coli* strains were collected (41 in 2012, 121 in 2013, 17 in 2014, 17 in 2015 and 9 in 2016). During these years, ESBL producers were detected in 10 (24.4%), 12 (9.9%), 4(23.5%), 6 (35.3%), and 3 (33.3%) of the isolates, respectively. Resistance profiles of the 35 ESBL producing and 29 non-ESBL producing isolates to all 15 antimicrobials tested are shown in detail in supplementary Table S1. Resistance rates to classes of antimicrobials commonly used for treating feline and canine *E. coli* UTIs are summarized in Table 1.

Overall, 46 (71.9%) of the isolates were MDR (88.6% of the ESBL-producers, and 51.7% of the non-ESBL producers, respectively). The presence of bla_{ESBL} genes was confirmed and the genes were identified in 35 phenotypic ESBL producers as $bla_{CTX-M-15}$ (n=17/48.6% of the bla_{ESBLs}), $bla_{CTX-M-1}$ (n=10/28.6%), $bla_{CTX-M-55}$ (n=4/11.4%), $bla_{CTX-M-14}$ (n=3/8.6%), and $bla_{CTX-M-27}$ (n=1/2.9%). Except for one $bla_{CTX-M-1}$, all bla_{ESBLs} were detected in canine isolates. Overall, 51 (79.7%) of the isolates were resistant to nalidixic acid, and 47 thereof were ciprofloxacin resistant (Table 1 and supplementary Table S1). Among the isolates displaying ciprofloxacin resistance, all revealed chromosomal mutations that result in amino acid substitutions in GyrA and ParC (Table 2 and supplementary Table S1). The plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr* and *qnrB* were detected in 17 (26.6% of all isolates), and one (1.6%) isolate, respectively, and were restricted to canine isolates (Table 3 and supplementary Table S1). The plasmid-mediated azithromycin resistance gene *mph(A)* was found in 13 (20.3%) of the isolates. The majority (n= 15/88.2%) of the 17 isolates with plasmid-mediated fluoroquinolone resistance and the majority (n= 34/69.4%) of the 49 isolates with chromosomally mediated quinolone resistance were ESBL producers (Table 3). Only 4 (6.3%) isolates were non-drug resistant (NDR) (supplementary Table S1).

Phylogenetic groups and ST

As shown in Table 3, the most prevalent phylogenetic group was C (n=19 /29.7% of all isolates), followed by group B2 (n=15 /23.4%), B1 (n=10/15.6%), group A (n=9/14%), group F (n=8/12.5%), and group D (n=3/4.7%). None of the isolates belonged to phylogenetic group E or clade I. Among feline isolates, phylogenetic group B2 predominated (n=7 /53.8% of all feline isolates), whilst among canine isolates, the majority (n=18/35.3%) belonged to group C. MLST assigned the majority (n=39/60.9%) of the isolates to 9 different clonal complexes (CC): CC10 (n=3), CC12 (n=2), CC23 (n=17), CC73 (n=5) and CC131 (n=5), CC156 (n=1), CC354 (n=2), CC469 (n=1), and CC648 (n=3).

Twenty-six different STs were identified, the three most common represented by ST410 (n=16/25%), ST131, and ST73 (both n=5/7.8%). Less frequently occurring STs included ST533 (n=4/6.3%), and ST361, ST744 and ST648 (each n=3/4.7%, respectively). All other STs were represented by one or two isolates only (Table 3 and supplementary Table S1).

Two new STs were detected, which however were not assigned numerical designations by the *E. coli* MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Figure 1 illustrates eBURST showing relationships between the isolates. The prevalence of the most common ST, ST410, was variable, declining from 50% in 2012 to 1% in 2015, with an increase to 33.3% observed for 2016. Of the 64 isolates, a total of 10 (15.6%) belonged STs frequently associated with human UTI (ST73 and ST131).

The MDR, ESBL- and non-ESBL producers were distributed throughout phylogroups A, B1, B2, C, D and F.

Distribution of VF genes

Overall, 47 (73.4%) of the isolates tested positive for one or more of the VF genes (Table 3). Among the 64 isolates, the prevalence of individual VF genes was 53.1% for *fyuA,* 48.4% for *traT,* 34.4% for *yfcv* and PAI, 23.4% for *hlyA*, 20.3% for *papAH*, and 18.8% for *papEF*.

The overall median aggregate VF score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3-7), followed by isolates from phylogenetic group F (3/ 2,9/1-4). Isolates assigned to phylogenetic group D scored 2/2/2-2, and isolates of phylogenetic group A scored 1/1,8/ 0-6. The overall median aggregate VF scores were lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3).

Among the most common STs in this study, ST131 harboured the highest number of VF (7/6,4/4-7), followed by ST73 (4/4,6/4-7), and ST648 (4/3,7/3-4). Of the most prevalent ST in this study, ST410 harboured the lowest number of VF (0/0,3/0-2).

VF scores were higher in NDR (4/3.8/3-4) than in MDR (1/1,9/0-7), higher in non-ESBL producing isolates (3/3/0-7) than in ESBL producers (1/1,7/0-7), and higher in isolates that did not harbour *mph(A)* (2/2,6/0-7), than those that harboured *mph(A)* (1/1,1/0-4).

Discussion

In this study, we assessed antimicrobial resistance, phylogenetic background, and virulence profiles of ESBL producing, and non-ESBL producing UPEC isolated from cats and dogs admitted to the veterinary clinic of the University of Zürich, Switzerland. In veterinary as in human medicine, antimicrobials are the cornerstone of treatment of bacterial UTI. Sulfamethoxazole/trimethoprim, amoxicillin/clavulanate, quinolones and chloramphenicol are antimicrobial compounds that are recommended for the treatment of feline and canine UTI due to infection with *E. coli* (Barsanti, 2012). Our data show that infection management may be compromised by the high rate of resistance to these drugs, similar to the situation /as described for UPEC in humans (Gibreel et al., 2012; Nicolle 2013; Nolan et al., 2015; Lavigne et al. 2016). Furthermore, of the 205 isolates from our strain collection, 35 (17%) were ESBL producers and the annual prevalence ranged from 9.9% to 35.3%. This is a remarkable increase compared to the prevalence of 7.5% ESBL producers among UPEC isolated from companion animals at our institution between 2010–2011 (Huber et al., 2013), and poses a threat to the efficacy of third-generation cephalosporins approved for use in veterinary medicine, such as cefovecin (Stegemann et al., 2006). Further, the high prevalences of 26.6% *aac(6')-Ib-cr* and 13.6% *mph(A)* among the UPEC analysed in this study contrast noticeably with the 2.3% and 13.6%, respectively, observed for these genes in UPEC from human primary care patients in Switzerland (Nüesch-Inderbinen et al., 2017), and indicate that companion animals, particularly dogs, may serve as reservoirs for *E. coli* harbouring these resistance genes.

In this study, we identified the phylogenetic groups based on the new Clermont method which enabled, besides the phylogenetic groups A, B1, B2, and D, the identification of groups C and F among the feline and canine UPEC. Our results show that in accordance to previous reports, UPEC isolates from cats frequently belonged to group B2 (Wagner et al., 2014; Liu et al., 2015). However, overall, phylogroup C had the highest prevalence, a finding which is in contrast to previous studies of UPEC in animals as well as in humans that indicate the

predominance of extraintestinal, pathogenic group B2 (Barsanti, 2012; Gibreel et al., 2012). Data on the prevalences of phylogroups C, E, F and clade I among UPEC are rare, but a recent study shows that among human isolates, approximately 25% belong to these groups (Iranpour et al., 2015). Since 42.2% of the isolates belonged to the newly described phylogroups C and F, further studies are needed to provide a more refined understanding of the prevalences of phylogenetic groups among *E. coli* isolates of animal and human origin.

Antimicrobial resistant as well as susceptible UPEC in humans are frequently associated with specific clonal complexes such as CC69, CC73, CC95, ST127, and CC131 (Lau et al., 2008; Gibreel et al., 2012). Some of these CCs or STs, such as CC73, ST127 and CC131 have also been described as etiological agents of UTI in companion animals (Johnson et al., 2008a; Johnson et al., 2009). Corresponding well with reports on human UPEC (Bengtsson et al., 2012), we found 80% of the *E. coli* ST131 isolates to ESBL producers, and all of the ST73 isolates to be non-ESBL producers. Overall, our data specified that 15.6% of UPEC from cats and dogs belong to STs commonly associated with UTI in humans and harbour urovirulence genes that are identical to those found in human isolates (Gibreel et al., 2012). Other STs found in this study, such as ST648 and ST410 are also associated with extraintestinal disease in humans and animals, but not specifically with UTI (Ewers et al., 2014; Falgenhauer et al. 2016; Schaufler et al. 2016; Timofte et al., 2016). Therefore, although major UPEC clones described in human infection were not predominant, such strains exhibit reciprocal zoonotic potential, and should be considered a threat to pet owners and companion animals alike.

The high prevalence of ST410 in ESBL-producing UPEC from dogs has been observed previously in Switzerland (Huber et al., 2013). This ST, assigned to phylogroup C, displayed inverse association of VF scores with MDR and with plasmid-mediated resistance to third-generation beta-lactams, aminoglycosides and macrolides. This phenomenon was observed among other isolates belonging to phylogenetic groups A and C, suggesting that selective pressure, rather than virulence may be the driving force behind the high prevalence of ST410 among canine UPEC. However, the panel of VFs selected for this study was limited in number and represents only a subset of known VFs. Other determinants of virulence may have been missed that may have shown a different tendency. Nevertheless, the high prevalence of isolates belonging to phylogenetic groups A, B1 and C indicate that the commensal flora of companion animals may play an important role in the aetiology of UTI.

The results of this study illustrate the need for further observation of *E. coli* causing disease in companion animals, as well as the prudent use of antimicrobials in veterinary medicine, including the use of third generation cephalosporins, fluoroquinolones and macrolides.

Conclusions

In conclusion, our findings showed that feline and canine UPEC population was characterized by a high prevalence of MDR. ESBLs producing UPEC were detected almost exclusively among canine isolates. Phylogenetic groups B2 and F were associated more frequently with VF than other groups. In this study, group C was the most predominant phylogenetic group and displayed the lowest number of VF. MLST revealed a diverse clonal structure, and detected STs that may pose an infectious threat to humans. The most frequent MLST was ST410, which was characterized by a high prevalence of plasmid-mediated resistance genes *bla*CTX-M-15, *aac(6')-Ib-cr* and *mph(A),* and a low aggregate VF score.

These results increase our current knowledge of the phenotypic and genotypic characteristics of feline and canine UPEC, and may be advantageous for future infection management and prevention.

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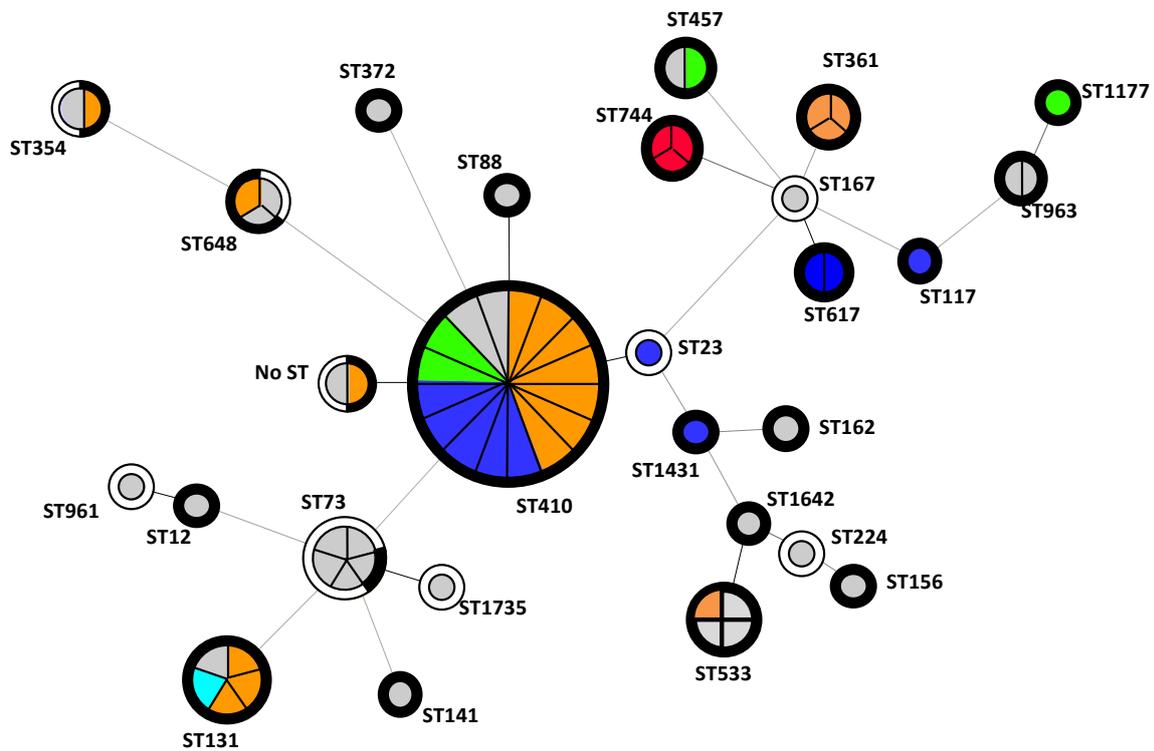
Figure 1: MLST-based eBURST diagram depicting non-ESBL and ESBL producing UPEC isolated from cats and dogs. Each ST is represented by a circle, the size of which correlates to the frequency of the ST. Pie parts represent the number of strains within one ST. Exact numbers of isolates per ST are given in Table 3 and supplementary Table S1. Black outer rings show percentage of dog-derived strains from dogs, white outer rings show percentage of strains from cats. Isolates that tested negative by PCR for bla_{ESBL} genes are coloured grey. Isolates harbouring $bla_{CTX-M-1}$ are depicted in blue, $bla_{CTX-M-14}$ in red, $bla_{CTX-M-15}$ in orange, $bla_{CTX-M-27}$ in turquoise, $bla_{CTX-M-55}$ in green.

Table 1: Resistance rates of feline and canine UPEC to classes of antimicrobials used in the management of *E. coli* UTI in cats and dogs.

	Antimicrobial class						
	ß-lactams		Folic acid synthesis inhibitors	Quinolones			
Host (n=64)	Ampicillin/ clavulanic acid	Cefotaxime	Sulfamethoxazol/ trimpethoprim	Naidixic acid	Ciprofloxacin	Chloramphenicol	Nitrofurantoin
Cats (n=13)	5 (38.5%)	5 (38.5%)	3 (23.1%)	7 (53.8%)	4 (30.8%)	4 (30.8%)	0 (0%)
Dogs (n=51)	22 (43.1%)	38 (74.5%)	30 (58.8%)	44 (86.3%)	43 (84.3%)	14 (27.5%)	2 (3.9%)

Table 2: Amino acid substitutions in the QRDR of 41 ciprofloxacin resistant UPEC isolates from cats and dogs.

	QRDR					
	gyrA			*parC*		
Host (n=41)	Ser83→Leu (%)	Asp87→Asn (%)	Asp87→Tyr (%)	Ser80→Ile (%)	Glu84→Val (%)	Glu84→Gly (%)
Cats (n=4)	4 (100)	3 (75)	1 (25)	4 (100)	0 (0)	1 (25)
Dogs (n=43)	43 (100)	39 (90.7)	3 (7)	43 (100)	4 (9.3)	4 (9.3)

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 3: Genotypic characteristics of feline and canine UPEC

Host	No. Isolates	Phylogenetic group	ST	CC	*papAH*	*papEF*	*yfcv*	*hlyA*	*fyuA*	*traT*	PAI	Plasmid-mediated resistance gene(s)
Cat	1	A	167	10	-	-	-	-	+	+	-	*mph(A)*
Dog	1	A	617	10	+	+	-	+	+	+	+	*bla*CTX-M-1
Dog	1	A	617	10	+	+	-	-	+	+	+	*bla*CTX-M-1
Dog	2	A	361	–	-	-	-	-	-	-	-	*aac(6')-Ib-cr, bla*CTX-M-15*, mph(A)*
Dog	1	A	361	–	-	-	-	-	-	+	+	-	*aac(6')-Ib-cr, bla*CTX-M-15*, mph(A)*
Dog	1	A	744	–	-	-	-	-	-	-	+	-	*bla*CTX-M-14*, mph(A)*
Dog	2	A	744	–	-	-	-	-	-	-	-	-	*bla*CTX-M-14
Dog	1	B1	156	156	-	-	-	-	+	-	-	-
Dog	1	B1	162	469	-	-	-	-	+	+	-	-
Cat	1	B1	224	–	-	-	-	-	-	+	-	-
Dog	1	B1	361	–	-	-	-	-	-	+	-	-
Dog	1	B1	533	–	-	-	-	-	-	+	-	*bla*CTX-M-15*, mph(A)*
Dog	2	B1	533	–	-	-	-	-	-	+	-	-
Dog	1	B1	1431	–	-	-	-	+	+	+	-	*bla*CTX-M-1
Dog	1	B1	1642	–	-	-	-	-	-	-	-	*mph(A)*
Cat	1	B1	Nd	nd	-	-	-	-	-	+	-	-
Dog	1	B2	12	12	+	+	+	+	+	-	+	-
Cat	3	B2	73	73	-	-	+	+	+	-	+	-
Cat	1	B2	73	73	+	+	+	+	+	+	+	-
Dog	1	B2	73	73	-	-	+	+	+	-	+	-
Dog	2	B2	131	131	+	+	+	+	+	+	+	*aac(6')-Ib-cr, bla*CTX-M-15
Dog	1	B2	131	131	+	+	+	+	+	+	+	*bla*CTX-M 15
Dog	1	B2	131	131	-	-	+	-	+	+	+	*bla*CTX-M-27*, mph(A)*
Dog	1	B2	131	131	+	+	+	+	+	+	+	-
Cat	1	B2	141	–	+	+	+	+	+	-	+	-
Cat	1	B2	961	–	+	+	+	+	+	-	+	-
Dog	1	B2	372	–	-	-	+	-	+	-	+	-
Cat	1	B2	1735	–	+	+	+	+	+	-	+	-

Table 3-continued

Host	No. Isolates	Phylogenetic group	ST	CC	*papAH*	*papEF*	*yfcv*	*hlyA*	*fyuA*	*traT*	PAI	Plasmid-mediated resistance gene(s)
Dog	1	C	88	12	+	-	-	-	+	+	-	-
Cat	1	C	23	23	-	-	-	-	+	-	-	$bla_{CTX-M-1}$
Dog	5	C	410	23	-	-	-	-	-	-	-	$aac(6')$-Ib-cr, $bla_{CTX-M-15}$,
Dog	5	C	410	23	-	-	-	-	-	-	-	$bla_{CTX-M-1}$
Dog	2	C	410	23	-	-	-	+	-	-	-	$aac(6')$-Ib-cr, $bla_{CTX-M-55}$, $mph(A)$
Dog	1	C	410	23	+	-	-	-	+	-		$aac(6')$-Ib-cr, $bla_{CTX-M-15}$, $mph(A)$
Dog	1	C	410	23	-	-	-	-	-	-	-	$aac(6')$-Ib-cr, $bla_{CTX-M-15}$, $mph(A)$
Dog	1	C	410	23	-	-	-	+	-	-	-	$aac(6')$-Ib-cr
Dog	1	C	410	23	-	-	-	-	-	-	-	$aac(6')$-Ib-cr
Dog	1	C	nd	nd	-	-	-	+	-	-	-	$aac(6')$-Ib-cr, $bla_{CTX-M-15}$, $mph(A)$, $qnrB$
Dog	2	D	963	–	-	-	-	-	+	+	-	-
Dog	1	D	1177	–	-	-	-	-	+	+	-	$bla_{CTX-M-55}$
Dog	1	F	354	354	-	-	+	-	-	+	-	$bla_{CTX-M-15}$
Cat	1	F	354	354	-	+	+	-	-	+	-	-
Dog	1	F	457	–	-	-	+	-	-	+	+	$bla_{CTX-M-55}$
Dog	1	F	457	–	-	-	+	-	-	+	+	-
Dog	1	F	648	648	-	-	+	-	+	+	+	$bla_{CTX-M-15}$
Dog	1	F	648	648	-	-	+	-	-	+	+	-
Cat	1	F	648	648	-	-	+	-	+	+	+	-
Dog	1	F	117	–	-	-	-	-	-	+	-	$bla_{CTX-M-1}$

Abbreviations: *aac(6')-Ib-cr,* aminoglycoside 6'-N-acetyltransferase variant; *bla*, ß-lactamase gene; CC, clonal complex; *fyuA*, ferric yersiniabactin uptake protein gene; *hlyA*, α-haemolysin gene; *mph(A)*, macrolide 2'-phosphotransferase gene; PAI, right-hand terminus of pathogenicity island; *papAH* and *papEF*, p-fimbrial adhesion genes; *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.

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