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Genome analysis of *Enterobacteriaceae* with non-wild type susceptibility to third-generation cephalosporins recovered from diseased dogs and cats in Europe

Line PEPIN-PUGET¹, Farid EL GARCH^{2,3}, Xavier BERTRAND^{1,4}, Benoit VALOT^{4,5}, Didier HOCQUET^{1,4,5*} on behalf of the ComPath Study Group

¹ *Hygiène Hospitalière, CHU Besançon, France*

² *Vétoquinol SA, Scientific division, Lure, France*

³ *ComPath Study Group, Bruxelles, Belgique*

⁴ *Université de Bourgogne Franche-Comté, UMR CNRS Chrono-environnement, Besançon, France*

⁵ *Bioinformatique et big data au service de la santé, UFR Santé, Université de Bourgogne Franche-Comté, Besançon, France'*

Corresponding author at : UMR CNRS 6249 Chrono-environnement, Université de Bourgogne Franche-Comté, France. Email address: dhocquet@chu-besancon.fr (D. Hocquet)

Key words: whole-genome sequencing; bacterial pathogens; animals; resistance; antibiotics

Abstract

Background. Extended-spectrum- β -lactamases (ESBL) and plasmid-mediated cephalosporinases (pAmpC)-producing *Enterobacteriaceae* isolates are now reported worldwide in humans, animals, and in the environment. We identified the determinants of resistance to β -lactams and associated resistance genes as well as phylogenetic diversity of 53 ESBL- or pAmpC-producing *Enterobacteriaceae* isolated from dogs and cats in Europe.

Materials/methods. Of a collection of 842 *Enterobacteriaceae* isolates that were recovered in 2013 and 2014 from 842 diseased and untreated dogs and cats, for 242 ampicillin or amoxicillin resistant isolates (MIC \geq 16 mg/L), cefotaxime (CTX) and ceftazidime (CAZ) MICs were determined. Isolates with CTX and/or CAZ MIC \geq 1 mg/L (n=63) were selected, and their genomes were fully sequenced using Illumina Technology. Genomic data were explored to identify the resistance determinants, the plasmid incompatibility groups, and the sequence types (STs). Plasmid location of *bla*_{ESBL} and *bla*_{AmpC} was evaluated for all isolates based on the co-localization of resistance and plasmid incompatibility group genes on the same contig. Phylogenetic trees were constructed using core-genome MLST.

Results. Of the 63 sequenced isolates, 53 isolates harboured a *bla*_{ESBL} or *bla*_{AmpC} gene. Ten CTX and/or CAZ non-wild type isolates had neither *bla*_{ESBL} nor *bla*_{AmpC}. Among the 63 isolates, 44 (69.8%) were *Escherichia coli*, 11 (17.5%) were *Klebsiella pneumoniae*, and 8 (12.7%) were *Proteus mirabilis*. Fifty-one (80.9%) isolates originated from dogs and 12 (19.1%) from cats. Isolates were sampled from urinary tract (n=36), skin and soft tissue (n=22) and respiratory tract infections (n=5). Thirty-two isolates (32/53, 60.4%) carried *bla*_{ESBL} genes, including *bla*_{CTX-M-15} (n=12), *bla*_{CTX-M-14} (n=6), *bla*_{CTX-M-1} (n=5), *bla*_{CTX-M-2} (n=3), *bla*_{CTX-M-27} (n=3), *bla*_{SHV-28} (n=4), *bla*_{SHV-12} (n=2), and *bla*_{VEB-6} (n=1). Four isolates of *K. pneumoniae* had both *bla*_{CTX-M-15} and *bla*_{SHV-28}. Twenty-one isolates (21/53, 39.6%) carried genes encoding pAmpC, including *bla*_{CMY-2} (n=19) and *bla*_{DHA-1} (n=2). Thirteen *E. coli* isolates harboured both *bla*_{ESBL} or *bla*_{AmpC} genes and plasmids of incompatibility groups IncIB (9/13), IncII (8/13), and IncFII (6/13). In addition to the reduced susceptibility to CTX and/or CAZ, reduced susceptibility or evidence of acquired resistance to at least one other relevant class of antibiotics was observed for all 63 isolates.

E. coli isolates clustered in 23 STs, including B2 virulent clones from humans such as ST131 (n=5), *K. pneumoniae* isolates mostly clustered in 3 STs: ST11 (n=4), ST307 (n=3), and ST16 (n=2). Phylogenetic analysis identified the spread of *E. coli* ST131 *bla*_{CTX-M-27}, and of *K. pneumoniae* ST307 harboring *bla*_{CTX-M-15} and *bla*_{SHV-28} or ST11 *bla*_{CTX-M-15}.

Conclusions. We report here a 6.3% prevalence of ESBL/pAmpC producing *Enterobacteriaceae* in diseased dogs and cats. This EU survey confirms that dogs and cats can be infected with epidemic multidrug resistant clones that may also spread in humans.

1. Introduction

Spread of extended-spectrum β -lactamases (ESBLs) and plasmid-mediated cephalosporinases (pAmpC)-producing *Enterobacteriaceae* is an important global public health concern (Canton et al., 2012; Rozwandowicz et al., 2018). During the last decades, the incidence of human ESBL/pAmpC-producing *Enterobacteriaceae* infections increased globally both in hospitals and in the community (Chong et al., 2018). Concomitantly, ESBL/pAmpC-producing *Enterobacteriaceae* have emerged worldwide in livestock, in food of animal origin and in companion animals (Bogaerts et al., 2015; Hordijk et al., 2013; Michael et al., 2017), enhancing the risk of spread of *bla*_{ESBL} and *bla*_{AmpC} genes from non-human sources and implying a potential risk of transmission of these bacteria or of resistance genes to humans from these new reservoirs (Pomba et al., 2017). The close contact between household pets and people offers favorable conditions for transmission. To illustrate the risk of human-pet transmission of bacteria or resistance genes in only one of the European industrial countries, the presence of 11.5 million cats and 6.9 million dogs in German households underlines the importance of these “family members” (Ohr, 2014).

Additional data are needed to clarify the epidemiology and the public health burden of these ESBL- and pAmpC-producing isolates from non-human reservoirs (Dorado-Garcia et al., 2018). The Executive Animal Health Study Center (CEESA) manages a monitoring program (ComPath) to survey antimicrobial susceptibility of bacterial pathogens isolated from dogs and cats suffering from respiratory tract infection (RTI), urinary tract infection (UTI) and skin and soft tissue infection (SSTI) in Europe (de Jong et al., 2013). As part of the ComPath program, our study aimed to identify determinants of resistance to β -lactams and associated resistance genes as well as phylogenetic diversity of *Enterobacteriaceae* isolates with non-wild type susceptibility to third-generation cephalosporins (3GC) recovered from diseased and untreated dogs and cats in Europe.

2. Material and methods

2.1. Bacterial isolates

Samples were collected in 2013 and 2014 from 842 diseased dogs and cats as a part of the CEESA Compath II program in 12 European countries (Fig. 1): Belgium, Czech Republic, France, Germany, Hungary, Italy, The Netherlands, Poland, Spain, Sweden, Switzerland, and the United Kingdom. Local laboratories in each country were responsible for recovery and identification of bacterial isolates, as stated previously (de Jong et al., 2013). Samples were collected by veterinary practitioners in their daily practice. One sample per animal was taken and diseased dogs and cats were untreated with any antimicrobial in the previous four weeks. Chronically-diseased animals were excluded. Only one animal per household, breeder, or kennel was included (de Jong et al., 2013).

2.2. Bacterial identification of isolates

On receipt in the central laboratory (IHMA Sarl Europe, Monthey, Switzerland) bacterial isolates were plated on tryptic soy agar (TSA) 5% sheep blood agar (Becton Dickinson, Franklin Lakes, NJ), then incubated at 35°C overnight in normal atmosphere (de Jong et al., 2013). Bacterial re-identification of all 3GC non-wild type susceptible isolates was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) on Microflex LT (Bruker Daltonics, Bremen, Germany). Acquired bacterial spectra were analyzed in the MALDI Biotyper 3.0 software 114 with the database version 3.1.2.

2.3. Antimicrobial susceptibility testing

Susceptibility to a panel of 14 to 20 antimicrobials commonly used to treat companion animals from UTI, SSTI and RTI was conducted at a central laboratory. The activity of antimicrobials was tested by agar microdilution against the *Enterobacteriaceae* isolates as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013). For all isolates with ampicillin or amoxicillin MIC \geq 16 mg/L, the MICs of cefotaxime (CTX) and ceftazidime (CAZ) were determined by broth microdilution according to CLSI (CLSI, 2013). *Enterobacteriaceae* with non-wild type susceptibility to 3GC (CTX and/or CAZ MIC \geq 1 mg/L) were selected for further analysis.

2.4. Genome sequencing analysis

Bacterial DNA was extracted with QIAamp DNA mini kit (Qiagen) and quantified using fluorometric analysis (Qubit® dsDNA BR Assay Kit, Invitrogen). Genomes were sequenced with Illumina NextSeq 500 (V2) to obtain 150-bp reads with at least 90x coverage. SPAdes (version 3.12) assembled bacterial genomes *de novo* from raw reads. Genomic data were explored to identify the resistance determinants (ResFinder ; 2019-01-04), the plasmid incompatibility groups (PlasmidFinder ; 2019-01-24), and the sequence types (STs ; 2019-01-10). Nucleotide and deduced amino acid sequences and chromosomal mutations of promoters and attenuators of *bla*_{AmpC} genes were compared to GenBank (AY899338.1) for *E. coli* isolates. Plasmid location of *bla*_{ESBL} and *bla*_{AmpC} was evaluated for all isolates based on the co-localization of resistance and plasmid incompatibility group genes on the same contig. STs were determined *in silico* using the database from the Center for Genomic Epidemiology (www.genomicepidemiology.org). The population structure of the three species included in the study (*E. coli*, *K. pneumoniae* and *P. mirabilis*) had been determined using a genome-wide approach. We used a homemade tool (<https://github.com/bvalot/wgMLST>) for a core genome MLST analysis (cgMLST) based on an allelic comparison of 1,861 genes for *E. coli*, 2,114 genes for *K. pneumoniae*, and 2,548 genes for *P. mirabilis*. Phylogenetic trees were constructed on the SNP present in these genes using Bayesian approach (MrBayes 3.2.6) with GTR+G model. *E. coli* isolates were sorted into phylogroups using the *in silico* tool (Beghain et al., 2018) to evaluate the virulence of the isolates (Picard et al., 1999). All the Read Archives will be available on NCBI under the Bioproject number PRJNA575837 at the time of the publication of the manuscript.

3. Results

Figures 1A, 1B, and 1C gather the genotypic and phenotypic data of the *E. coli*, *K. pneumoniae* and *P. mirabilis* isolates with the non-wild type susceptibility to 3GC, respectively, with Figure 1D giving the color signification.

3.1. Bacterial collection and antimicrobial susceptibility testing

Among the ComPath collection of 842 *Enterobacteriaceae* isolates recovered during 2013-2014, we selected all the isolates (n=63, 7.5%) with MICs of CTX and/or CAZ ≥ 1 mg/L. The distribution of the species is given in Table 1. Fifty-two (82.6%) isolates originated from dogs and 11 (17.5%) from cats. Isolates were recovered from UTI (n=36), SSTI (n=22) and RTI (n=5).

In addition to the reduced susceptibility to 3GC, reduced susceptibility or evidence of acquired resistance to at least one other relevant class of antibiotics was observed for all 63 isolates. They were non-susceptible to fluoroquinolones (n=48), to gentamicin (n=19), or to trimethoprim/sulfamethoxazole (n=27)(Supplemental Table 1).

3.2. Identification of bla_{ESBL} and bla_{AmpC}

Among the 63 *Enterobacteriaceae* isolates sequenced, 53 harboured either bla_{ESBL} or bla_{AmpC} . The details of these genes is given in Table 1. Thirty-two (60.4%) isolates carried bla_{ESBL} genes, including 17 bla_{CTX-M} of group 1 (twelve $bla_{CTX-M-15}$ and five $bla_{CTX-M-1}$), nine bla_{CTX-M} of group 9 (six $bla_{CTX-M-14}$ and three $bla_{CTX-M-27}$) and three bla_{CTX-M} of group 2 ($bla_{CTX-M-2}$). Twenty-one (39.6%) isolates carried an AmpC-encoding genes, including 19 bla_{CMY-2} . Ten 3GC non-wild type isolates had neither bla_{ESBL} nor bla_{AmpC} genes. Among them, three isolates of *E. coli* had an identical transversion at position -32 in the -35 box of the *ampC* promoter.

ESBL- and pAmpC-producing isolates were retrieved from nine participating countries. Forty-four (83.2%) isolates originated from dogs and nine (17.3%) from cats. Isolates producing ESBL or pAmpC enzymes were recovered from pets suffering from UTIs (n=30), SSTIs (n=20) and RTIs (n=3).

Table 1. ESBL and pAmpC-encoding genes of 63 *Enterobacteriaceae* isolates with non-wild type susceptibility to third-generation cephalosporins retrieved from diseased and untreated dogs and cats in Europe (2013-2014).

<i>bla</i> _{ESBL} and <i>bla</i> _{AmpC} gene(s)	Species				Isolates (<i>n</i>) per animal species	
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	Total	Cats	Dogs
<i>bla</i> _{CMY-2}	13	-	6	19	4	15
<i>bla</i> _{CTX-M-15}	5	3	-	8	1	7
<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-28}	-	4	-	4	-	4
<i>bla</i> _{CTX-M-14}	6	-	-	6	1	5
<i>bla</i> _{CTX-M-1}	3	2	-	5	2	3
<i>bla</i> _{CTX-M-2}	3	-	-	3	-	3
<i>bla</i> _{CTX-M-27}	3	-	-	3	-	3
<i>bla</i> _{SHV-12}	2	-	-	2	-	2
<i>bla</i> _{DHA-1}	1	1	-	2	-	2
<i>bla</i> _{VEB-6}	-	-	1	1	-	1
Unidentified	8	1	1	10	3	7
Total (%)	44 (69.8)	11 (17.5)	8 (12.7%)	63	11	52

3.3. Plasmid characterization

Forty-eight of the 63 isolates (76.2%) harbored one or several plasmids (1 to 8). Thirteen *E. coli* isolates harbored both ESBL- and pAmpC-encoding genes, together with genes of plasmids which incompatibility groups are given in Table 2. No *K. pneumoniae* neither *P. mirabilis* isolates had co-localization of resistance and plasmid incompatibility group genes on the same contig.

Table 2. Co-localization of resistance and plasmid incompatibility group genes on the same contig for 13 *E. coli* isolates from diseased and untreated dogs and cats in Europe (2013-2014) with non-wild type susceptibility to third-generation cephalosporins.

Isolate ID	Plasmid(s) per isolate (n)	Plasmid incompatibility group											<i>bla</i> _{ESBL/AmpC} gene on the same contig			
		ColI56	Col440	ColpVC	IncB/O/K/Z	IncFIA	IncFIB	IncFIC	IncFII	IncHII	IncI1	IncI2		IncQ1	IncX1	p0111
EC_20	1															<i>bla</i> _{CMY-2}
EC_21	6															<i>bla</i> _{CMY-2}
EC_32	1															<i>bla</i> _{CMY-2}
EC_39	3															<i>bla</i> _{CMY-2}
EC_40	4															<i>bla</i> _{CMY-2}
EC_62	3															<i>bla</i> _{CMY-2}
EC_71	5															<i>bla</i> _{CMY-2}
EC_75	6															<i>bla</i> _{CMY-2}
EC_59	3															<i>bla</i> _{CTX-M-1}
EC_12	7															<i>bla</i> _{CTX-M-14}
EC_44	1															<i>bla</i> _{CTX-M-14}
EC_76	7															<i>bla</i> _{CTX-M-14}
EC_24	4															<i>bla</i> _{SHV-12}
Total		3	1	2	4	4	9	1	8	1	8	1	3	1	1	

3.4. Phylogenetic groups and Multilocus sequence typing

E. coli clustered in 23 STs. Among the 22 *E. coli* harboring a *bla*_{ESBL} gene, twelve belonged to a virulent extraintestinal phylogroup B2 (ST131, n=4; ST73, n=1), phylogroup D (ST405, n=1; ST362, n=2; ST38, n=2) and phylogroup F (ST457, n=1). The remaining ten isolates belonged to phylogroups A and B1. Among the 14 *E. coli* isolates harboring *bla*_{AmpC} genes, ten belonged to phylogroup B2 (ST131, n=1; ST372, n=2; ST80, n=1; ST625, n=1), phylogroup D (ST38, n=1; ST973, n=1) and phylogroup F (ST354, n=2). The remaining four isolates were sorted in the phylogroups A, B1 or C. One B2 virulent isolate of *E. coli* ST131 harbored both *bla*_{CTX-M-15} and plasmid of incompatibility group IncFII genes. Three *E. coli* isolates belonging to the virulent phylogroup D (ST405, n=1; ST362, n=2) harbored both *bla*_{CTX-M-14} and plasmid of incompatibility group IncFII.

K. pneumoniae harboring *bla*_{ESBL} clustered in a few STs. The epidemic high-risk international clones (i) ST11, represented by four isolates, among which two harbored *bla*_{CTX-M-15} and two harbored *bla*_{CTX-M-1} and (ii) ST15, represented by one isolate having both *bla*_{CTX-M-15} and *bla*_{SHV-28}. The emerging high-

risk clone ST307 was represented by three isolates harboring *bla*_{CTX-M-15} and *bla*_{SHV-28}. The sole *K. pneumoniae* isolate with the pAmpC-encoding gene *bla*_{DHA-1} belonged to ST16.

3.5. Phylogenetic analysis

Phylogenetic analysis along with the plasmid and resistance determinant profiles identified the spread in pets of ESBL-producing *Enterobacteriaceae* that are epidemic in humans (Figure 1A and 1B; Supplemental Table 1). We found that two UK dogs shared a strain of *E. coli* ST131 that carried *bla*_{CTX-M-27} (isolates EC_43 and EC_45). Besides, two isolates of *E. coli* ST362 carrying *bla*_{CTX-M-14} (isolates EC_12 and EC_76) were found in one dog and one cat with UTI from Spain (Figure 1A). Finally, the clone *K. pneumoniae* ST307 harboring *bla*_{CTX-M-15} and *bla*_{SHV-28} (isolates KP_13 and KP_14) was shared by two UTI-suffering dogs from Czech Republic (Figure 1B; Supplemental Table 1).

4. Discussion

The prevalence of *Enterobacteriaceae* infecting dogs and cats and harboring *bla*_{ESBL} or *bla*_{AmpC} genes was relatively low (6.3%), in line with those found by other studies (3.8-15.3%) in Europe [(reviewed by (Ewers et al., 2012)]. Similarly, the 2008-2010 European surveillance program ComPath I found a prevalence of ESBL- or pAmC-producing *Enterobacteriaceae* of 3.6% in infected dogs and cats (Bogaerts et al., 2015). This prevalence can reach 40.4% in China, where it mirrors that measured in humans (Ewers et al., 2012; Quan et al., 2017).

The resistance to 3GC in our collection is mostly associated with the dissemination of CTX-M-type enzymes, which are now the most prevalent ESBLs worldwide. Although less frequently reported than *bla*_{CTX-M}, the genes *bla*_{AmpC} (and in particular *bla*_{CMY-2}) now spread among *E. coli* and *Proteus* spp. (Marques et al., 2018). Here, the pAmpC genotype was found in 21 *Enterobacteriaceae* isolates out of 63 (33.3%), essentially related to *bla*_{CMY-2} as previously reported in companion animals (Bogaerts et al., 2015; Bortolami et al., 2019; Melo et al., 2018). In the *E. coli* isolates of our collection, *bla*_{CMY-2} was co-localized with various plasmid genes (Table 2), especially IncII plasmids which are widespread in *E. coli* isolates of animal origin (Carattoli, 2013; Hansen et al., 2016). This large proportion of CMY-2-producing *Enterobacteriaceae* is worrisome since (i) this enzyme has a stronger β -lactamase activity and (ii) CMY-2 isolates also harbored other resistance mechanisms, such as porin deficiency leading to the non-enzymatic resistance to carbapenems (Goessens et al., 2013; Jacoby, 2009).

Similarly to what is observed in humans, CTX-M-type enzymes is the most common ESBL type circulating in companion animals and humans and share a similar epidemiology probably due to the close contact between pets and their owners (Canton et al., 2008). The CTX-M-type ESBLs CTX-M-1, -14, and -15 are commonly found in companion animals (Ewers et al., 2012). Their geographic distribution mirrors that of the human enzymes, with CTX-M-1 predominating in Africa and Europe, CTX-M-14 in Asia and North America, and CTX-M-15 worldwide (Rubin and Pitout, 2014). In our work, the low prevalence of *E. coli* isolates harboring *bla*_{CTX-M-1} confirms data found in a study in United Kingdom (Bortolami et al., 2019) but was lower than that retrieved in clinical samples from

Germany and from The Netherlands (Dierikx et al., 2012; Schink et al., 2013). CTX-M-1 has been found to dominate the distribution of ESBL/pAmpC in pigs, veal calves, and cattle while CTX-M-15 dominate in humans (Dorado-Garcia et al., 2018). The low distribution of CTX-M-1 in the present collection that contrasted with that of CTX-M-15 may reflect the living environment (*i.e.* more urban than rural) of the companion animals sampled in our study.

The prevalence of the emerging ST131 C1/H30R *bla*_{CTX-M-27} clade (also known as C1-M27 sublineage) increased in humans (Birgy et al., 2017; Matsumura et al., 2016). A recent study that examined ESBL-producing isolates from livestock, humans, companion animals, food, and the environment between 2009 and 2016 in Germany found that the prevalence of *bla*_{CTX-M-27} increased exclusively in isolates of human origin (Ghosh et al., 2017). In our study, we found two *E. coli* C1-M27 (isolates EC_43 and EC_45) in two UK dogs with SSTI, with identical pattern of plasmid and resistance genes.

E. coli ST131 *bla*_{CTX-M-15} is a worldwide pandemic clone in humans, but more rarely reported in infected companion animals (Nicolas-Chanoine et al., 2014). In our study, two *E. coli* ST131 producing CTX-M-15 were found, one from UTI sample and one from RTI sample, both from the same clinic in Czech Republic, but with different plasmid and resistance pattern (Supplemental Table 1).

The emergence and clonal spread of *K. pneumoniae* have also been reported in recent years. *Klebsiella* spp. isolates from companion animals have been well documented worldwide and in particular in European countries (Donati et al., 2014; Ewers et al., 2014; Hidalgo et al., 2013; Marques et al., 2019b; Poirel et al., 2013; Wohlwend et al., 2015). As previously found, *bla*_{CTX-M-15} genotype dominated in the eleven *K. pneumoniae* isolates of this work (Ewers et al., 2014). Here, we report the spread of *K. pneumoniae* ST307 (isolates KP_13, KP_14 from Germany and KP_79 from Spain) and the occurrence of clones ST11 and ST15, with those three clones also spreading in human (Marques et al., 2019b; Villa et al., 2017). Two recent studies evidenced shared *K. pneumoniae* strains between companion animals, humans, and the environment including isolates producing CMY-2-like, DHA-1-like or/and CTX-M-type enzymes (Hong et al., 2019; Marques et al., 2019a). However, the

transmission routes between pets and their owner need to be clarified using longitudinal studies exploited by WGS.

Fewer data have documented the occurrence of resistance genes to β -lactams in *P. mirabilis* isolated from companion animals (Marques et al., 2018). However, we confirmed here that most 3GC non-wild type *P. mirabilis* isolates produced the plasmid-borne cephalosporinase CMY-2.

The ten isolates with a non-wild type susceptibility to 3GC without *bla*_{BLSE} or *bla*_{AmpC} genes had low level of resistance to CAZ or CTX (≤ 0.25 -4 mg/L). Such levels of resistance to 3GC can be due to a reduced impermeability or to the overproduction of the intrinsic AmpC cephalosporinase. Hence, three isolates of *E. coli* (belonging to independent STs) had a transversion (T→A) that creates the *E. coli* consensus -35 box TTGACA, responsible to the overexpression of AmpC and the resistance to 3GC (Tracz et al., 2007).

5. Conclusion

Here, we assessed the prevalence and molecular diversity of ESBL- and pAmpC-encoding genes of *Enterobacteriaceae* isolates infecting dogs and cats and found pathogenic and resistant clones that also spread in humans. This might suggest that dogs and cats can be either the source or the target of such antibiotic resistant pathogens, which transmission routes still have to be deciphered with longitudinal studies applying WGS.

Figure legend

Figure 1. Phylogeny, source and resistance data of *E. coli* (A), *K. pneumoniae* (B) and *P. mirabilis* (C) isolates with a non-wild type susceptibility to third-generation cephalosporins isolated from diseased and untreated dogs and cats in Europe (2013-2014). The Bayesian phylogenetic trees of *E. coli*, *K. pneumoniae*, and *P. mirabilis* were based on 1 861, 2 114, and 2 548 core genes, respectively. We used the reference genomes of *Shigella dysenteriae*, *Klebsiella variicola*, and *Proteus vulgaris* as outgroups for *E. coli*, *K. pneumoniae*, and *P. mirabilis* collections, respectively. The names of the countries of isolation were abbreviated following the international code ISO 3166-1 alpha-2. UTI, urinary tract infection; SSTI, skin and soft tissue infection; RTI, respiratory tract infections. The MIC are given in mg/l.

References

- Beghain, J., Bridier-Nahmias, A., Le Nagard, H., Denamur, E., Clermont, O., 2018. ClermonTyping: an easy-to-use and accurate *in silico* method for *Escherichia* genus strain phylotyping. *Microb genom* 4, e000192.
- Birgy, A., Bidet, P., Levy, C., Sobral, E., Cohen, R., Bonacorsi, S., 2017. CTX-M-27-producing *Escherichia coli* of sequence type 131 and clade C1-M27, France. *Emerg Infect Dis* 23, 885.
- Bogaerts, P., Huang, T.D., Bouchahrouf, W., Bauraing, C., Berhin, C., El Garch, F., Glupczynski, Y., 2015. Characterization of ESBL- and AmpC-producing *Enterobacteriaceae* from diseased companion animals in Europe. *Microb Drug Resist* 21, 643-650.
- Bortolami, A., Zendri, F., Maciuca, E.I., Wattret, A., Ellis, C., Schmidt, V., Pinchbeck, G., Timofte, D., 2019. Diversity, virulence, and clinical significance of extended-spectrum β -lactamase- and pAmpC-producing *Escherichia coli* from companion animals. *Front Microbiol* 10, 1260.
- Canton, R., Gonzalez-Alba, J.M., Galan, J.C., 2012. CTX-M Enzymes: Origin and diffusion. *Front Microbiol* 3, 110.
- Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., Coque, T.M., 2008. Prevalence and spread of extended-spectrum β -lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 14 Suppl 1, 144-153.
- Carattoli, A., 2013. Plasmids and the spread of resistance. *Int J Med Microbiol* 303, 298-304.
- Chong, Y., Shimoda, S., Shimono, N., 2018. Current epidemiology, genetic evolution and clinical impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect Genet Evol* 61, 185-188.
- CLSI, 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard – Fourth Edition. Vol. 33 n° 07. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- de Jong, A., Thomas, V., Klein, U., Marion, H., Moyaert, H., Simjee, S., Valle, M., 2013. Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. *Int J Antimicrob Agents* 41, 403-409.

- Dierikx, C.M., van Duijkeren, E., Schoormans, A.H., van Essen-Zandbergen, A., Veldman, K., Kant, A., Huijsdens, X.W., van der Zwaluw, K., Wagenaar, J.A., Mevius, D.J., 2012. Occurrence and characteristics of extended-spectrum- β -lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *J Antimicrob Chemother* 67, 1368-1374.
- Donati, V., Feltrin, F., Hendriksen, R.S., Svendsen, C.A., Cordaro, G., Garcia-Fernandez, A., Lorenzetti, S., Lorenzetti, R., Battisti, A., Franco, A., 2014. Extended-spectrum- β -lactamases, AmpC β -lactamases and plasmid mediated quinolone resistance in *Klebsiella* spp. from companion animals in Italy. *PLoS One* 9, e90564.
- Dorado-Garcia, A., Smid, J.H., van Pelt, W., Bonten, M.J.M., Fluit, A.C., van den Bunt, G., Wagenaar, J.A., Hordijk, J., Dierikx, C.M., Veldman, K.T., de Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T., Velthuis, A.G., Heuvelink, A., Gonggrijp, M.A., van Duijkeren, E., van Hoek, A., de Roda Husman, A.M., Blaak, H., Havelaar, A.H., Mevius, D.J., Heederik, D.J.J., 2018. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. *J Antimicrob Chemother* 73, 339-347.
- Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum β -lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clin Microbiol Infect* 18, 646-655.
- Ewers, C., Stamm, I., Pfeifer, Y., Wieler, L.H., Kopp, P.A., Schonning, K., Prenger-Berninghoff, E., Scheufen, S., Stolle, I., Gunther, S., Bethe, A., 2014. Clonal spread of highly successful ST15-CTX-M-15 *Klebsiella pneumoniae* in companion animals and horses. *J Antimicrob Chemother* 69, 2676-2680.
- Ghosh, H., Doijad, S., Falgenhauer, L., Fritzenwanker, M., Imirzalioglu, C., Chakraborty, T., 2017. *bla*_{CTX-M-27}-encoding *Escherichia coli* sequence type 131 lineage C1-M27 clone in clinical isolates, Germany. *Emerg Infect Dis* 23, 1754-1756.
- Goessens, W.H., van der Bij, A.K., van Boxtel, R., Pitout, J.D., van Ulsen, P., Melles, D.C., Tommassen, J., 2013. Antibiotic trapping by plasmid-encoded CMY-2 β -lactamase combined with

- reduced outer membrane permeability as a mechanism of carbapenem resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 57, 3941-3949.
- Hansen, K.H., Bortolaia, V., Nielsen, C.A., Nielsen, J.B., Schonning, K., Agero, Y., Guardabassi, L., 2016. Host-specific patterns of genetic diversity among Inc11-I γ and IncK plasmids encoding CMY-2 β -lactamase in *Escherichia coli* isolates from humans, poultry meat, poultry, and dogs in Denmark. *Appl Environ Microbiol* 82, 4705-4714.
- Hidalgo, L., Gutierrez, B., Ovejero, C.M., Carrilero, L., Matrat, S., Saba, C.K., Santos-Lopez, A., Thomas-Lopez, D., Hoefler, A., Suarez, M., Santurde, G., Martin-Espada, C., Gonzalez-Zorn, B., 2013. *Klebsiella pneumoniae* sequence type 11 from companion animals bearing ArmA methyltransferase, DHA-1 β -lactamase, and QnrB4. *Antimicrob Agents Chemother* 57, 4532-4534.
- Hong, J.S., Song, W., Park, H.M., Oh, J.Y., Chae, J.C., Shin, S., Jeong, S.H., 2019. Clonal spread of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* between companion animals and humans in South Korea. *Front Microbiol* 10, 1371.
- Hordijk, J., Wagenaar, J.A., van de Giessen, A., Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Kant, A., Mevius, D., 2013. Increasing prevalence and diversity of ESBL/AmpC-type β -lactamase genes in *Escherichia coli* isolated from veal calves from 1997 to 2010. *J Antimicrob Chemother* 68, 1970-1973.
- Jacoby, G.A., 2009. AmpC β -lactamases. *Clin Microbiol Rev* 22, 161-182, Table of Contents.
- Marques, C., Belas, A., Aboim, C., Cavaco-Silva, P., Trigueiro, G., Gama, L.T., Pomba, C., 2019a. Evidence of sharing of *Klebsiella pneumoniae* strains between healthy companion animals and cohabiting humans. *J Clin Microbiol* 57.
- Marques, C., Belas, A., Franco, A., Aboim, C., Gama, L.T., Pomba, C., 2018. Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. *J Antimicrob Chemother* 73, 377-384.

- Marques, C., Menezes, J., Belas, A., Aboim, C., Cavaco-Silva, P., Trigueiro, G., Telo Gama, L., Pomba, C., 2019b. *Klebsiella pneumoniae* causing urinary tract infections in companion animals and humans: population structure, antimicrobial resistance and virulence genes. *J Antimicrob Chemother* 74, 594-602.
- Matsumura, Y., Pitout, J.D., Gomi, R., Matsuda, T., Noguchi, T., Yamamoto, M., Peirano, G., DeVinney, R., Bradford, P.A., Motyl, M.R., Tanaka, M., Nagao, M., Takakura, S., Ichiyama, S., 2016. Global *Escherichia coli* sequence type 131 clade with *bla*_{CTX-M-27} gene. *Emerg Infect Dis* 22, 1900-1907.
- Melo, L.C., Oresco, C., Leigue, L., Netto, H.M., Melville, P.A., Benites, N.R., Saras, E., Haenni, M., Lincopan, N., Madec, J.Y., 2018. Prevalence and molecular features of ESBL/pAmpC-producing *Enterobacteriaceae* in healthy and diseased companion animals in Brazil. *Vet Microb* 221, 59-66.
- Michael, G.B., Kaspar, H., Siqueira, A.K., de Freitas Costa, E., Corbellini, L.G., Kadlec, K., Schwarz, S., 2017. Extended-spectrum β -lactamase-producing *Escherichia coli* isolates collected from diseased food-producing animals in the GERM-Vet monitoring program 2008-2014. *Vet Microb* 200, 142-150.
- Nicolas-Chanoine, M.H., Bertrand, X., Madec, J.Y., 2014. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 27, 543-574.
- Ohr, R., 2014. Pet study – Pet Ownership as Economical Factor, the economic significance of pet ownership in Germany, Göttingen.
- Picard, B., Garcia, J.S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J., Denamur, E., 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. *Infect Immun* 67, 546-553.
- Poirel, L., Nordmann, P., Ducroz, S., Boulouis, H.J., Arne, P., Milleman, Y., 2013. Extended-spectrum β -lactamase CTX-M-15-producing *Klebsiella pneumoniae* of sequence type ST274 in companion animals. *Antimicrob Agents Chemother* 57, 2372-2375.
- Pomba, C., Rantala, M., Greko, C., Baptiste, K.E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M.A., Pyorala, S., Ruzauskas, M., Sanders, P., Teale, C., Threlfall, E.J., Kunsagi, Z., Torren-Edo,

- J., Jukes, H., Torneke, K., 2017. Public health risk of antimicrobial resistance transfer from companion animals. *J Antimicrob Chemother* 72, 957-968.
- Quan, J., Zhao, D., Liu, L., Chen, Y., Zhou, J., Jiang, Y., Du, X., Zhou, Z., Akova, M., Yu, Y., 2017. High prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in community-onset bloodstream infections in China. *J Antimicrob Chemother* 72, 273-280.
- Rozwandowicz, M., Brouwer, M.S.M., Fischer, J., Wagenaar, J.A., Gonzalez-Zorn, B., Guerra, B., Mevius, D.J., Hordijk, J., 2018. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. *J Antimicrob Chemother* 73, 1121-1137.
- Rubin, J.E., Pitout, J.D., 2014. Extended-spectrum β -lactamase, carbapenemase and AmpC producing *Enterobacteriaceae* in companion animals. *Vet Microb* 170, 10-18.
- Schink, A.K., Kadlec, K., Kaspar, H., Mankertz, J., Schwarz, S., 2013. Analysis of extended-spectrum- β -lactamase-producing *Escherichia coli* isolates collected in the GERM-Vet monitoring programme. *J Antimicrob Chemother* 68, 1741-1749.
- Tracz, D.M., Boyd, D.A., Hizon, R., Bryce, E., McGeer, A., Ofner-Agostini, M., Simor, A.E., Paton, S., Mulvey, M.R., 2007. *ampC* gene expression in promoter mutants of cefoxitin-resistant *Escherichia coli* clinical isolates. *FEMS Microbiol Lett* 270, 265-271.
- Villa, L., Feudi, C., Fortini, D., Brisse, S., Passet, V., Bonura, C., Endimiani, A., Mammina, C., Ocampo, A.M., Jimenez, J.N., Doumith, M., Woodford, N., Hopkins, K., Carattoli, A., 2017. Diversity, virulence, and antimicrobial resistance of the KPC-producing *Klebsiella pneumoniae* ST307 clone. *Microb Genom* 3, e000110.
- Wohlwend, N., Endimiani, A., Francey, T., Perreten, V., 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.

Figure 1A

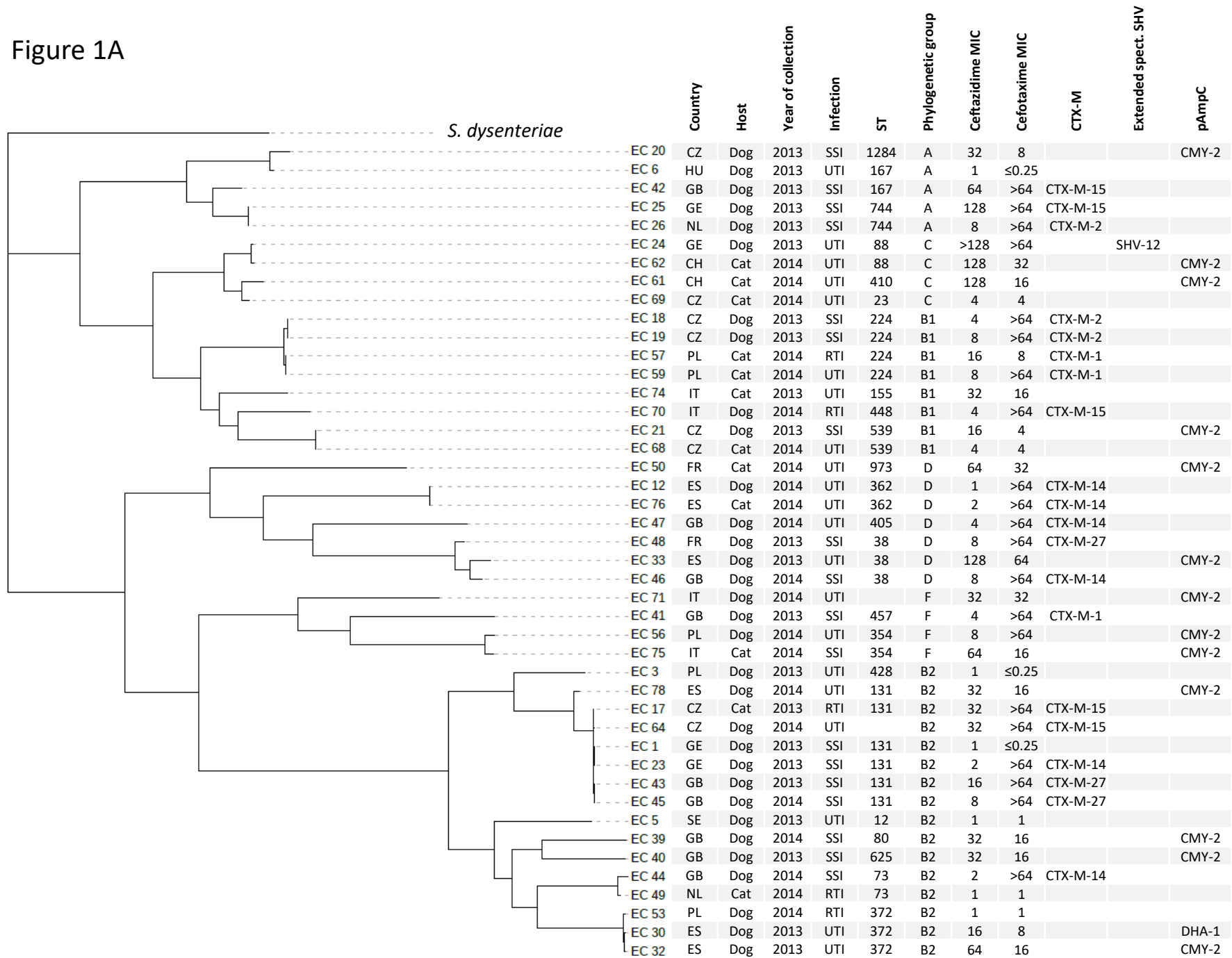


Figure 1B

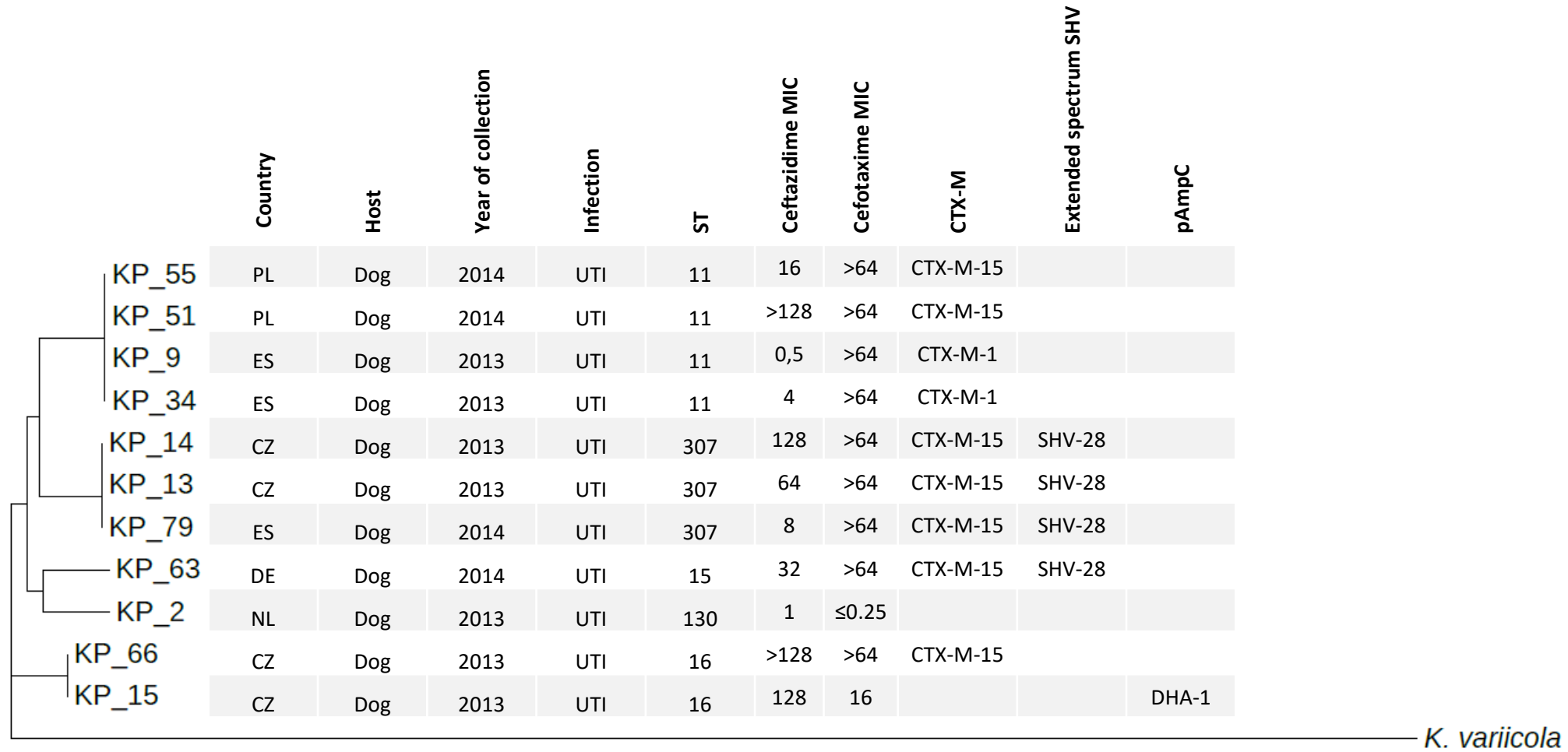
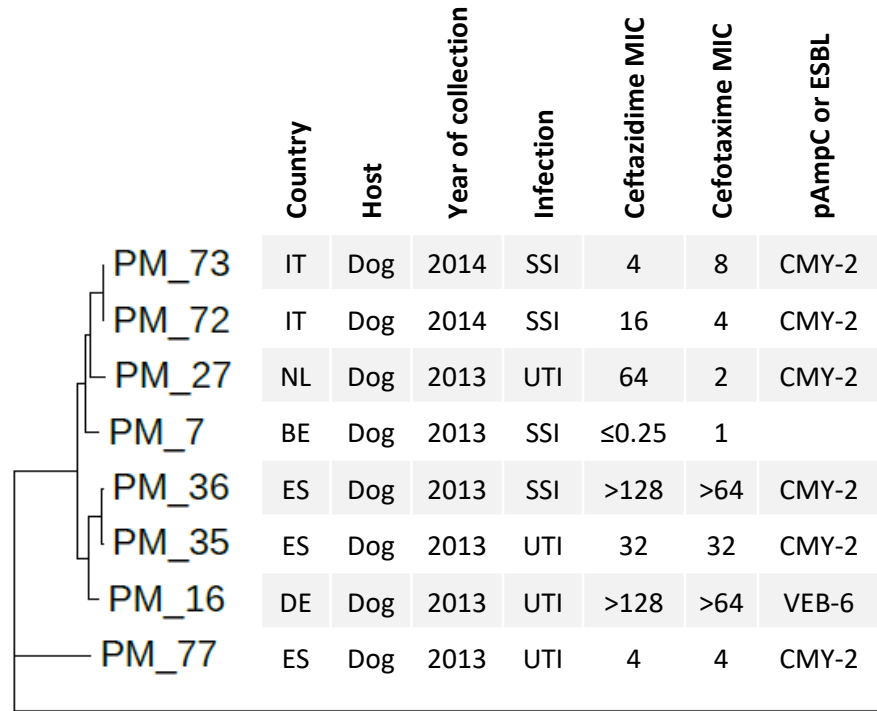


Figure 1C



P. vulgaris