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Resistance plasmids in ESBL-encoding *Escherichia coli* isolates from humans, dogs and cats

Resistenzplasmide in ESBL-kodierenden Escherichia coli-Isolaten aus Proben von Menschen, Hunden und Katzen

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Summary

We characterized ESBL-producing *Escherichia coli* isolates from diseased dog, cat and human sources for their plasmid content. Plasmids with different Inc groups and combinations of resistance genes were detected in these isolates. The pan-genome of the plasmid-associated genes was found to be large, indicating diversity of the gene pool among the plasmids. No commonly occurring plasmids with similar gene content in isolates from dog, cats and humans were detected.

Keywords: Antibiotic resistance, Inc group, CTX-M, spread of resistances, ESBL

Zusammenfassung

Aus klinischen Proben von erkrankten Hunden, Katzen und Menschen wurden ESBL-produzierende *Escherichia coli* isoliert, charakterisiert und auf ihren Plasmidgehalt untersucht. Eine große Anzahl an unterschiedlichen Inc-Gruppen, Plasmiden und Resistenzgenen konnte in den untersuchten Isolaten nachgewiesen werden. Es fand sich ein großes Pan-Genom der Plasmid-assoziierten Gene, was darauf hinweist, dass Diversität innerhalb der Plasmide vorhanden ist. Es konnten keine verbreitet vorkommenden Plasmide mit ähnlichen Genen in Isolaten aus Hund, Katze und Mensch gefunden werden.

Schlüsselwörter: Antibiotikaresistenz, Inc-Typ, CTX-M, Übertragung von Resistenzen, ESBL

Introduction

Extended-spectrum beta-lactamases (ESBL), especially of type CTX-M, have become the most prevalent extended-spectrum beta-lactamases in Gram-negative bacteria of both humans and animals, with CTX-M-1 and CTX-M-15 associated with the bulk of isolates (Ewers et al., 2012). ESBL-producing Enterobacteriaceae strains have been isolated from different sources including healthy and diseased humans (Mshana et al., 2009; Valenza et al., 2014;), companion animals (Dierikx et al., 2012; Ewers et al., 2012) as well as sick (Schink et al., 2013) and healthy farm animals (Wieler et al., 2011; Friese et al., 2013). A possible zoonotic-like transmission of resistance has been proposed (Ewers et al., 2012; Franiek et al., 2012; Dierikx et al., 2013). Therefore, handling of those animals might be a risk for colonization of humans with ESBL isolates, particularly of pet owners, veterinarians and animal keepers (Meyer et al., 2012).

The transfer of ESBL resistance genes from bacterial donors to recipients is most often mediated by plasmids (Bush, 2010; Carattoli, 2013). However, integration of CTX-M-15 into the genome of *Salmonella enterica* serotype Concord (Fabre et al., 2009), as well as *Escherichia coli* ST131 (Andersen et al., 2013; Hirai et al., 2013) has been described.

As plasmids are epidemiologically important vectors in ESBL transfer, the study of their structure and function is of great interest. Since interspecies transfer of plasmids through conjugation is possible, an identical plasmid can be found in different genetic backgrounds. Transfer of the same plasmids from animal to human bacteria or vice versa has been shown in broilers and broiler farmers in the Netherlands (Dierikx et al., 2013). Therefore, plasmid transfer from bacteria of companion animal holders to bacteria of companion animals or vice versa appears to be possible. However, studies addressing this issue in more detail have not been published

yet. Other studies have used multi-locus sequence typing to examine the relationships between isolates from human and companion animals (see for example, Ewers et al., 2012). Here we focus on the role of plasmids in mediating zoonotic transmission. We report the result of an initial analysis of plasmid contents in a small set of ESBL-encoding *Escherichia coli* isolates from both diseased companion animals and humans.

Material and Methods

A subset of 20 ESBL-producing *E. coli* isolates was selected from a pool of 390 beta-lactamase-producing Enterobacteriaceae from human (group one) and animal (companion animals, horses and farm animals; group two) samples. Human isolates (n=183) were taken from the isolate collection of the Institute of Medical Microbiology, Giessen. They were selected during routine screening for ESBL-producing Enterobacteriaceae at the University hospital in Giessen between 2009 and 2010. Animal isolates (n=207) were collected during a survey for aerobic gram-negative bacteria growing on MacConkey agar supplemented with cefotaxime (1 mg/l) among animal patients presented at the veterinary clinics in Giessen between 2009 and 2011. For the present study, five CTX-M-1 and five CTX-M-15 harbouring *E. coli* isolates were exemplarily chosen. In case of group two, only isolates from cats and dogs were included.

Isolates were tested for possible ESBL production by double disc synergy testing (DDST). The test was carried out using ceftriaxone (30 µg), ceftazidime (30 µg) and amoxicillin/clavulanic acid (30 µg) (231635, 231633, 231629 Becton Dickinson AG, GER). Antibiotic susceptibility testing was performed using the VITEK®2 XL system (bioMérieux, FRA) with AST N117 cards (22290, bioMérieux, FRA) and Liofilchem® MIC Test Strips containing ertapenem, cefepime, chloramphenicol and nalidixic acid (921570, 921260, 920750, 921320, Liofilchem® s.r.l., ITA), respectively. Results were evaluated according to CLSI guidelines for human pathogens (CLSI, 2012). Genotyping of ESBL alleles by PCR using specific oligonucleotide primers for the bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes (Kiratisin et al., 2008; Gröbner et al., 2009) was done as described previously (Mshana et al., 2009). PCR-positive isolates were sequenced to identify the encoded ESBL allele precisely. Sequencing was performed using the automated sequencer ABI Prism® 3100 (Applied Biosystems, USA). The blastn algorithm of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was used for database searches. Following the classification of Doumith et al. (2012) identification of *E. coli* phylogenetic groups A, B1, B2 and D was accomplished by PCR analysis targeting genes *chuA, yjaA, gadA* and the DNA fragment TSPE4.C2. For multilocus sequence typing (MLST) analysis internal fragments of the seven housekeeping genes *adk, fumC, gyrB, icd, mdh, purA, recA* were sequenced (Wirth et al., 2006). Sequence type (ST) and sequence type complex (STC) were assigned in compliance with the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli). Identification of the major plasmid incompatibility groups among *E. coli* was performed according to Carattoli et al. (2005). Primer pairs for Inc groups FIA, FIB, I1 and N were used. Bacterial genomic DNA was extracted, treated with S1-nuclease and analysed by pulsed field gel electrophoresis (Mshana et al., 2009) to determine quantity and sizes of plasmids present in each *E. coli* isolate.

Investigated *E. coli* isolates were submitted to next generation sequencing of whole genomes. Briefly, cells were grown at 37°C overnight in LB medium and total DNA was extracted using PureLink® Genomic DNA Kit (K182001, Life Technologies GmbH, GER). The sequencing library was prepared using Nextera XT Sample prep (FC-131-1096, FC-131-1001, Illumina Netherlands, NL) and sequencing was performed on a MiSeq instrument (Illumina Netherlands, NL). Raw reads were assembled into contigs using Spades (version 2.0 and 3.; Bankevich et al., 2012). KmerFinder (http://cge.cbs.dtu.dk/services/KmerFinder/) was used to identify an appropriate reference genome. KmerFinder is based on a number of co-occurring k-mers (16 base pair long nucleotide sequences) between the query genome and a genome in the reference database. The relevant reference was used for subsequent analysis. Mapping of all contigs against this reference was performed using MAUVE (Darling et al., 2010). Contigs that did not map to the respective reference chromosomes were then subjected to a blast search against the NCBI nucleotide collection (http://blast.ncbi.nlm.nih.gov/). Those contigs that produced strong hits with plasmid sequences were considered to be probable parts of plasmids and subsequently used to construct plasmid pseudo-genomes.

This set of data was then used for further analysis. To examine for the presence of resistance and virulence genes, we used Resfinder (Zankari et al., 2012) and VirulenceFinder (http://cge.cbs.dtu.dk/services/VirulenceFinder/), respectively. Incompatibility groups FII and FIC were assigned using PlasmidFinder (http://cge.cbs.dtu.dk/services/PlasmidFinder/).

Results

Resistance patterns of investigated *E. coli* isolates

The resistance pattern of the 20 investigated isolates showed distinct and identical combinations and some overlap in these combinations amongst companion animal and human isolates (see Table 1). Four patterns were found in both groups: Pattern #1 (AMP, FEP, CTX, CHL, CIP, NAL, SXT, TET) is found in a dog and a human outpatient isolate. Both of these strains harboured the CTX-M-1 allele and belonged to the same phylogenetic group B1. Pattern #2 (AMP, FEP, CTX, CAZ, CHL, CIP, GEN, NAL, SXT, TET) occurred in three isolates (dog and cat, phylogenetic group A; human/outpatient phylogenetic group B1). This pattern appeared to be restricted to CTX-M-15. Pattern #3 (AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET) was displayed by both CTX-M-1 and CTX-M-15 encoding isolates and was present in two dog isolates and three isolates from hospitalized humans. Pattern #6 was found in a cat isolate and in a human outpatient sample. Both isolates carried the CTX-M-1 allele, but were members of different phylogenetic groups.

Inc groups and plasmid content

Each isolate investigated harboured at least two plasmids (see Table 2), with two exceptions: V74, a canine isolate, harboured only one plasmid and H93 which was devoid of plasmids. In general, isolates from companion animals did not carry IncN plasmids; in contrast, we detected IncN plasmids in three isolates from hospital-

TABLE 1: *General characteristics and resistance phenotypes of ESBL-producing E. coli isolates from human and companion animal sources (n = 20)*

Isolate[a]	Origin	Source of isolation	Phylo-genetic group	ST type	bla_{CTX-M}	Resistance pattern[b]	Resistance pattern #
V63	Dog	intracavitary uterine content	B1	162	CTX-M-1	AMP, FEP, CTX, CHL, CIP, NAL, SXT, TET	1
V70	Dog	urinary catheter	A	361	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, GEN, NAL, SXT, TET	2
V74	Dog	semen sample	B1	617	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET	3
V76	Dog	throat swab	B1	1056	CTX-M-1	AMP, FEP, CTX, CHL, SXT, TET	4
V105	Dog	urine	A	3476	CTX-M-1	AMP, CTX, CHL, CIP, NAL, SXT	5
V143	Cat	stool sample	A	88	CTX-M-1	AMP, FEP, CTX, CHL, CIP, GEN, NAL, SXT, TET	6
V161	Dog	organ	A	New ST[c]	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET	3
V210	Cat	unknown	B2	New ST[c]	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, GEN, NAL, SXT, TET	2
V260	Dog	urine	D	131	CTX-M-15	AMK, AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET	7
V288	Dog	bronchoalveolar lavage	D	354	CTX-M-1	AMP, FEP, CTX, CHL, CIP, GEN, NAL, SXT, TET	8
H1	Human/outpatient	urine	B1	453	CTX-M-1	AMP, FEP, CTX, CHL, CIP, NAL, SXT, TET	1
H44	Human/outpatient	urine	B1	10	CTX-M-1	AMP, FEP, CTX, CHL, CIP, GEN, NAL, SXT, TET	6
H75	Human/hospitalized	urine	B2	131	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, GEN, SXT, TET	9
H92	Human/hospitalized	penule swab	A	10	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET	3
H93	Human/outpatient	urine	B1	156	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, GEN, NAL, TET	9
H115	Human/hospitalized	urine	A	744	CTX-M-1	AMP, FEP, CTX, CAZ, CHL, CIP; NAL, SXT, TET	3
H131	Human/hospitalized	urine	B2	131	CTX-M-1	AMP, FEP, CTX, CAZ, CHL, CIP, NAL, SXT, TET	3
H132	Human/outpatient	cervical smear	B2	131	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, NAL, SXT, TET	10
H139	Human/hospitalized	urine	A	88	CTX-M-1	AMP, FEP, CTX, CAZ, CHL, NAL, SXT, TET	10
H152	Human/outpatient	inguinal swab	B1	224	CTX-M-15	AMP, FEP, CTX, CAZ, CHL, CIP, GEN, NAL, SXT, TET	2

[a] V, veterinary isolates; H, human isolates
[b] AMK, amikacin; AMP, ampicillin; FEP, cefepime; CTX, cefotaxime, CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline
[c] "New ST" refers to novel sequence types which have not been assigned yet

TABLE 2: *Identified quantities, Inc groups and plasmid sizes of plasmids harboured by the investigated ESBL-producing E.coli isolates*

Isolate	Origin[a]	Source of isolate	Encoded ESBL	Identified Inc groups						Plasmids (quantity, size)[c]
				FIA	FIB	FIC[b]	FII[b]	I1	N	
V63	Dog	intracavitary uterine content	CTX-M-1		+	+	+			145 kb/96 kb
V70	Dog	urinary catheter	CTX-M-15	+	+		+			160 kb/95 kb/47 kb
V74	Dog	semen sample	CTX-M-15	+	+		+			160 kb
V76	Dog	throat swab	CTX-M-1		+	+	+	+		145 kb/110 kb/97 kb/30 kb
V105	Dog	urine	CTX-M-1		+	+	+	+		100 kb/80 kb
V143	Cat	stool sample	CTX-M-1		+	+	+			100 kb/80 kb/4 kb
V161	Dog	organ	CTX-M-15	+	+		+			160 kb/60 kb
V210	Cat	unknown	CTX-M-15	+	+		+			130 kb/110 kb/30 kb
V260	Dog	urine	CTX-M-15	+			+			200 kb/160 kb/3 kb
V288	Dog	bronchoalveolar lavage	CTX-M-1	+	+		+			110 kb/50 kb
H1	Human/outpatient	urine	CTX-M-1		+	+	+	+		300 kb/130 kb/100 kb/95 kb
H44	Human/outpatient	urine	CTX-M-1					+		100 kb/90 kb
H75	Human/hospitalized	urine	CTX-M-15	+			+			130 kb/50 kb
H92	Human/hospitalized	penule swab	CTX-M-15				+			200 kb/60 kb
H93	Human/outpatient	urine	CTX-M-15							No plasmid detected
H115	Human/hospitalized	urine	CTX-M-1		+				+	120 kb/30 kb
H131	Human/hospitalized	urine	CTX-M-1	+					+	90 kb/36 kb
H132	Human/outpatient	cervical smear	CTX-M-15		+		+	+		130 kb/90 kb
H139	Human/hospitalized	urine	CTX-M-1					+	+	100 kb/36 kb
H152	Human/outpatient	inguinal swab	CTX-M-15	+	+		+			140 kb/90 kb

[a] V, veterinary isolates; H, human isolates
[b] Inc Groups IncFII and IncFIC were defined using PlasmidFinder
[c] quantification and size determination by S1 nuclease restriction followed by pulsed-field gel electrophoresis (PFGE), kb= kilo base pairs

TABLE 3: *Distribution of antibiotic and detergent resistance genes in the investigated ESBL-producing isolates*

Isolate	Origin	Source of isolate	Beta-Lactam						Aminoglycoside										Fluoroquinolone	Macrolide		Phenicol			Sulphonamide			Tetracycline		Trimethoprim				Detergent
			bla$_{CTX-M-1}$	*bla*$_{CTX-M-15}$	*bla*$_{OXA-1}$	*bla*$_{TEM-1}$	*bla*$_{TEM-30}$	*bla*$_{TEM-84}$	*aac(3)-IIa*	*aac(3)-IId*	*aac(3)-IVa*	*aph(3')-Ic*	*aph(4)-Ia*	*aadA1*	*aadA2*	*aadA5*	*strA*	*strB*	*aac(6')Ib-cr*	*mph(A)*	*mef(B)*	*catA1*	*catB3*	*cmlA1*	*sul1*	*sul2*	*sul3*	*tet(A)*	*tet(B)*	*dfrA1*	*dfrA12*	*dfrA14*	*dfrA17*	*qacE delta*
V63	Dog	intracavitary uterine content	■			■										■	■	■				■				■			■				■	
V76[b]	Dog	throat swab	■														■	■								■		■	■					
V105[b]	Dog	urine	■													■	■	■								▒							■	
V143	Cat	stool sample	■								■		■	■			■	■		■					■				■					■
V288[c]	Dog	bronchoalveolar lavage	■		■	■				■				■		■	■	■		■		■			■	■			■				■	■
H1[b]	Human/outpatient	urine	■			■								■											▒	■			■	■				■
H44[b]	Human/outpatient	urine	■			■					■		■					▒								▒		■						
H115	Human/hospitalized	urine	■			■								■	■					■	■	■		■			■		■		■			
H131	Human/hospitalized	urine	■													■				▒					■			■					■	■
H139[b]	Human/hospitalized	urine	■			■								■			■	■		■						■		■		■				■
V70[c]	Dog	urinary catheter		■	■			■	■			■		■					■			■	▒		■			■		■				■
V74[c]	Dog	semen sample		■	■											■	▒	▒	■	■			▒		■	■			■				■	■
V161[c]	Dog	organ		■	■		■		■								■	■	■	■			▒			■		■				■		
V210[c]	Cat	unknown		■	▒		■		■								■	■	■	■			▒			■		■				■		
V260	Dog	urine		■	■											■			■	■			▒		■			■					■	■
H75	Human/hospitalized	urine		■	■	▒			■										■				▒					■						
H92	Human/hospitalized	penule swab		■	■	■									■		■	■	■	■			▒		■	■					■	■		■
H93[a]	Human/outpatient	urine		■	■				■										■				▒											
H132[b]	Human/outpatient	cervical smear		■		■											■	■		■					■	■		■					■	■
H152[c]	Human/outpatient	inguinal swab		■	■				■							■			■	▒			▒		■			■					■	■

[a] H93 showed no plasmids in S1 nuclease digestion, therefore the resistances found in this strain are presumably inserted into the genome
[b] IncI1 plasmid present
[c] combination of IncFIA, IncFIB and IncFII present
legend: white: gene not found, black: gene found, identity on nucleotide sequence level >98%, grey: gene found in part, only, but with high identity on nucleotide sequence level

ized humans. Similarly, IncI1 plasmids were more abundant in human than in companion animal isolates. In human isolates every single isolate harboured a different set of plasmid Inc groups. In isolates from companion animals we detected the combination of FIA, FIB and FII plasmids in five isolates. This combination was also observed in one human isolate. Combinations comprising FIB/FIC/FII and FIB/FIC/FII/I1 were each found twice in companion animals. The latter combination was also found in one human isolate.

Antibiotic and detergent resistance genes on plasmid contigs

Search for antibiotic resistance genes was performed using Resfinder on plasmidic contigs, analysis for detergent resistance gene *qacE* delta was performed using blastn. Table 3 provides an overview of resistance genes detected. At least eight classes of different antibiotic resistances were present in the strains investigated. Each strain harboured at least five different resistance genes. For better comparison, strains harbouring CTX-M-1 or CTX-M-15 were analysed separately. Only two isolates (V161, dog, V210, cat) showed an almost identical resistance gene pattern, indicating that these strains might harbour similar resistance cassettes. All CTX-M-15 encoding strains except two (V210, H132) harboured a combination of CTX-M-15, *aac(6')Ib-cr,* a deletion of *cat*B3 (deletion on the 3' end, 422 base pair length for all of the strains) and OXA-1. This combination resembled that of an insertion sequence present in plasmid pHg of *Klebsiella pneumoniae* strain ATCC BAA-2146, (accession number CP006662.1), pKDO1 of *K. pneumoniae* (accession number JX424423.1) and pEK516 of *E. coli* strain D (accession number EU935738.1). Isolates harbouring CTX-M-1 did not exhibit such combinations. In human isolates, a combination of CTX-M and TEM-1 appeared to be more often in CTX-M-1 than in CTX-M-15 harbouring isolates and more often in human than in companion animal isolates. All CTX-M-1-positive companion animal isolates also harboured *str*A and *str*B, while this gene pattern was seen in one human clinical isolate, only.

Virulence genes encoded on plasmid contigs

Only few plasmids harboured putative virulence genes (see Table 4). Commonly occurring genes included *iro*N (salmochelin, n=4 isolates), *iss* (increased serum survival protein encoded on plasmids like pAPEC-O1-ColBM, accession number DQ381420.1, n=4), *cba* (colicin M activity protein, n=1), *cma* (colicin B activity protein, n=1), *senB* (putative enterotoxin, n=1), *mcmA* (gene of

TABLE 4: *Virulence genes found in the investigated strains*

Isolate	Origin	Source of isolate	Encoded ESBL	Virulence factor[a]						
				iroN	*iss*	*mchF*	*cba*	*cma*	*senB*	*mcmA*
V63	Dog	intracavitary uterine content	CTX-M-1	+	+	+				
V70	Dog	urinary catheter	CTX-M-15							
V74	Dog	semen sample	CTX-M-15							
V76	Dog	throat swab	CTX-M-1	+	+	+				
V105	Dog	urine	CTX-M-1							
V143	Cat	stool sample	CTX-M-1	+	+	+				+
V161	Dog	organ	CTX-M-15							
V210	Cat	unknown	CTX-M-15							
V260	Dog	urine	CTX-M-15							
V288	Dog	bronchoalveolar lavage	CTX-M-1							
H1	Human/outpatient	urine	CTX-M-1	+	+	+				
H44	Human/outpatient	urine	CTX-M-1				+	+		
H92	Human/hospitalized	urine	CTX-M-15							
H75	Human/hospitalized	penule swab	CTX-M-15							
H93	Human/outpatient	urine	CTX-M-15							
H115	Human/hospitalized	urine	CTX-M-1							
H131	Human/hospitalized	urine	CTX-M-1							
H132	Human/outpatient	cervical smear	CTX-M-15						+	
H139	Human/hospitalized	urine	CTX-M-1							
H152	Human/outpatient	inguinal swab	CTX-M-15							

[a] identity on nucleotide sequence level >98%

microcin M synthesis operon, n=1) and *mchF* (gene of microcin H47 operon, n=4). We found that *iro*N, *iss* and *mch*F were always associated with the CTX-M-1 gene. In each strain these virulence genes were arranged in a conserved pattern resembling the situation found e. g. in plasmid pAPEC-1 of *E. coli* strain chi7122 (accession number CP000836.1).

Discussion

A major goal of this study was to identify similarities and differences in the plasmid content of ESBL encoding strains from *E. coli* isolates obtained from diseased companion animals and human patients. The main focus was on isolates encoding CTX-M-1 or CTX-M-15 as they are the most common occurring CTX-M alleles in human and companion animal samples. All bacterial isolates originated from a distinct geographical area in Germany and allowed comparison of the antibiotic resistance phenotypes and antibiotic resistance genes in these two populations.

Analysis of the resistance phenotypes showed in some cases similarities between human and companion animal isolates. The resistance phenotype itself -as expected- was not associated with plasmid content and Inc group pattern. Nevertheless, a relationship between Inc group and CTX-M subtype was detected since *E. coli* isolates containing IncI1 group plasmids harboured the CTX-M-1 gene in five of six cases. This finding corroborates the recent conclusion of other studies that IncI1 plasmids seem to be associated with spread of CTX-M-1 in human and poultry (Accogli et al., 2013). Similarly, the combination of FIA, FIB and FII was associated with the CTX-M-15 gene in five of six cases. This combination has also previously been noted by Carattoli et al. (2008).

In this small set of isolates we only found IncN group plasmids in human isolates. This is consistent with data reported in the literature. IncN plasmids have been shown to be present in *E. coli* from humans, cattle, horses, pigs and wild water birds (Dolejska et al., 2013), but not in dogs or cats. Recently, IncN plasmids have been reported in *Klebsiella pneumoniae* isolates from dogs (Donati et al., 2014).

Two CTX-M-15-positive *E. coli* strains isolated from a dog and a cat, respectively, harboured plasmids of different sizes albeit belonging to identical Inc groups. In addition, an almost identical resistance gene combination was observed for these strains. This suggests a common source of these plasmids or the transfer and integration of the same resistance gene cassettes into related plasmids.

Identical Inc group combinations were also observed among other subsets of isolates (Table 2; FIB/FIC/FII/I1: V76, V105, H1; FIA/FIB/FII: V161, V210, V288, H152). However, isolates of these subsets differed from each other in plasmid size as well as resistance genes patterns. Thus, it appears reasonable to assume that these isolates have evolved independently.

This study provides a first description of the types of plasmids and resistance patterns in a defined catchment area in Germany. Our data has limitations since we have examined only a small number of isolates from this distinct geographical region albeit from within the same time period. Nevertheless, they indicate the presence of distinct populations of plasmids in human and companion animal isolates. Studies are presently underway to validate these findings in a larger set of strains from the same isolation period. Further studies would address the plasmid content of isolates of companion animals and those derived from isolates obtained from their direct owners.

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Conflict of interest: The authors declare that no competing interests exist.

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