J Antimicrob Chemother 2010; **65**: 651–660
doi:10.1093/jac/dkq004 Advance publication 29 January 2010

Journal of Antimicrobial Chemotherapy

Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-β-lactamase-producing *Escherichia coli* among companion animals

Christa Ewers[1]*, Mirjam Grobbel[2], Ivonne Stamm[3], Peter A. Kopp[3], Ines Diehl[1], Torsten Semmler[1], Angelika Fruth[4], Janine Beutlich[5], Beatriz Guerra[5], Lothar H. Wieler[1] and Sebastian Guenther[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Philippstr. 13, 10115 Berlin, Germany; [2]Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany; [3]Vet Med Labor GmbH, Moerikestr. 28/3, 71636 Ludwigsburg, Germany; [4]National Reference Centre for Salmonella and Other Enteric Pathogens, Robert Koch Institute, Burgstr. 37, 38855 Wernigerode, Germany; [5]Department of Biological Safety, Federal Institute for Risk Assessment (BfR), Diedersdorfer Weg 1, 12277 Berlin, Germany

*Corresponding author. Tel: +49-30-20936153; Fax: +49-30-20936067; E-mail: ewers.christa@vetmed.fu-berlin.de

Received 14 October 2009; returned 18 November 2009; revised 29 December 2009; accepted 1 January 2010

Objectives: In view of the intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15 extended-spectrum β-lactamase (ESBL) in human clinical settings it would be of great interest to explore its existence in animals to unravel a possible reservoir function and the origin and transmission of this group of multiresistant strains.

Methods: A total of 177 clinical phenotypically ESBL-producing *E. coli* isolates, mainly obtained from companion animals with urinary tract infections, wound infections and diarrhoea, were collected in a veterinary diagnostic laboratory covering a European-wide service area. They were screened for molecular subtype O25b and multilocus sequence type 131. O25b-ST131 isolates were subsequently tested for ESBL types, and phenotypic and genotypic resistance determinants. Further characterization of the strains was performed by PFGE and virulence gene typing.

Results: Ten (5.6%) of 177 phenotypically ESBL-producing *E. coli* isolates, nine strains from dogs and one strain from a horse, were allocated to the B2-O25b-ST131 lineage. Nine of these isolates harboured a CTX-M-15-type β-lactamase enzyme while one strain possessed an SHV-12-type ESBL. Macrorestriction analysis revealed a cluster formation of six of the animal CTX-M-15-type ESBL-producing strains from five different European countries together with a human control strain constituting a group of clonally related strains at a similarity value of 87.0%.

Conclusions: Our findings demonstrate that the group of clonally related human B2-O25:H4-ST131 CTX-M-15-type ESBL-producing *E. coli* strains is present in companion animals from various European countries. This highlights the possibility of inter-species transmission of these multiresistant strains from human to animal and vice versa.

Keywords: antimicrobial resistance, dogs, cats, horses, MLST

Introduction

In recent years the emergence of CTX-M enzymes, a new group of plasmid-mediated extended-spectrum β-lactamases (ESBLs), in human and animal populations has gained worldwide attention, replacing classical TEM and SHV-type ESBLs in many countries.[1–3] Among >80 variants described in this group of enzymes, CTX-M-15 has been shown to be the most frequent one associated with human isolates from hospital and community settings worldwide, implying serious therapeutic challenges due to its ability to hydrolyse cefotaxime and ceftazidime.[1,4] The pandemic distribution of the CTX-M-15 enzymes in the past decade has been partly associated with the recent and fast global dissemination of a highly virulent ciprofloxacin-resistant clonally related group of *E. coli* characterized by their multilocus sequence type (MLST; ST131), phylogenetic group

(B2) and O type (O25:H4).[5–8] It has been shown that this group of ESBL-producing *E. coli* has emerged and disseminated in different continents with a major focus on human populations, mainly implicated in cases of urinary tract infection (UTI) and bacteraemia. In contrast, the most prevalent CTX-M variants in ESBL producers in animals and food of animal origin are currently CTX-M-1 and CTX-M-14, while CTX-M-15 ESBL-producing *E. coli* have only exceptionally been observed in the veterinary context.[2,3] There is one report about an animal source of a CTX-M-15 ESBL-producing B2-ST131-O25:H4 *E. coli*, namely a dog suffering from UTI,[9] indicating a possible entry of this emergent clone into the animal population and/or its underestimated role in veterinary medicine most probably due to less extensive investigations. Apart from this exceptional finding, so far no other strains from UTIs in dogs have been proven to represent the group of ST131 ESBL producers.[10,11]

To discern a possible spread of the pandemic human virulent *E. coli* clone B2-O25:H4-ST131 producing a CTX-M-15-type ESBL among the animal population, mainly companion animals, we investigated third-generation cephalosporin-resistant *E. coli* isolates collected in a veterinary diagnostic laboratory in Germany providing a European-wide service area. The identification of this pandemic clone among a larger collection of clinical isolates from animals and its similarity to human isolates sheds new light on the origin, transmission and possible reservoir of this group of multiresistant strains.

Materials and methods

Bacterial strains

A total of 177 phenotypically ESBL-positive, consecutive non-repetitive clinical strains of *E. coli* from eight European countries, mainly from Germany ($n=133$), but also from Italy ($n=13$), the Netherlands ($n=13$), France ($n=7$), Spain ($n=6$), Denmark ($n=2$), Austria ($n=2$) and Luxembourg ($n=1$), were collected in a veterinary diagnostic service laboratory in Germany from April 2008 to June 2009. Phenotypical verification of the bacterial species was confirmed by using the VITEK2 System (bioMérieux, Germany).

The isolates originated from dogs ($n=84$), cats ($n=31$), horses ($n=50$), cattle ($n=7$), guinea pigs ($n=2$) and one pygmy rabbit, pig and American Kestrel, respectively. The clinical isolates were associated with UTIs ($n=64$), urogenital tract infections such as metritis, cervicitis and mastitis ($n=10$), wound infection (WI; $n=54$), enteritis ($n=29$) and various other clinical diseases ($n=20$). Another five non-ESBL *E. coli* strains, previously typed as ST131 in our laboratory (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli), were included for comparative analysis. These strains were isolated from a pig (IMT13407) and a dog (RL322/96) with enteritis, a human patient with UTI (IMT9087), a healthy chicken (IMT15150) and a healthy human (IMT18399). The human B2-O25:H4-ST131 CTX-M-15 ESBL-producing *E. coli* strain DSM22664 from the German collection of microorganisms, isolated from a case of bloody enteritis, was used as a reference strain.

O typing

O types of all *E. coli* strains were initially determined with a recently described molecular approach based on allele-specific PCR, targeting the *rfbO25b* subgroup gene locus.[6] The determination of O and H antigens of *rfbO25b*-positive strains was subsequently carried out by conventional serotyping at the Robert Koch Institute (Wernigerode, Germany), the National reference centre for Salmonella and other enteric pathogens.

MLST and phylogenetic grouping

MLST determination was carried out as described previously.[12] Gene amplification and sequencing were performed by using primers specified on the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli) except for *adk* (FP 5′-TCATCATCTGCACTTTCCGC-3′; RP 5′-CCAGATCAGCGCGAA CTTCA-3′), *mdh* (FP 5′-AGCGCGTTCTGTTCAAATGC-3′; RP 5′-CAGGTTCAGAA CTCTCTCTGT-3′) and *gyrB*, for which only the reverse primer was newly designed (RP 5′-ATCAGGCCTTCACGCGCATC-3′). Sequences were analysed by the software package Ridom SeqSphere 0.9.19 (http://www3.ridom.de/seqsphere) and STs were computed automatically. The phylogenetic group of the *E. coli* strains was determined by the software Structure 2.3.X based on the concatenated sequences of the seven housekeeping genes used for MLST (http://pritch.bsd.uchicago.edu/structure.html).

Determination of phenotypic and genotypic resistance

Phenotypically, production of β-lactamases was confirmed by the method given in CLSI guideline M31-A3.[13] *E. coli* ST131 strains were further characterized with regard to ESBL enzymes and other resistance determinants as described previously, including isoelectric focusing (IEF) of the enzymes and PCR amplification of the respective genes.[14] The plasmid localization of bla_{SHV} and $bla_{CTX\text{-}M\text{-}15}$ genes as well as plasmid replicon typing was performed by Southern blotting and subsequent hybridization analyses using protocols and template strains described previously.[15,16] The presence of antibiotic resistance genes, such as *tet*(A), *tet*(B), *tet*(C), *sul1*, *sul2*, *strA*, *strB*, *aadA1-like*, *aacC4*, bla_{TEM}-like and bla_{SHV}, and those previously described to be associated with plasmids carrying the $bla_{CTX\text{-}M\text{-}15}$ genes, i.e. bla_{OXA} (OXA groups 1, 2 and 10), *acc(6′)-Ib* and the *qnrA*, *qnrB* and *qnrS* genes, was determined by PCR. Sequence analysis was subsequently performed on bla_{TEM}-like and *acc(6′)-Ib* amplicons for identification of bla_{TEM} types and the *acc(6′)-Ib-cr* variant.[17–23] The presence of the *intI1* and *intI2* genes, encoding class 1 and 2 integrases, respectively, was determined by PCR using primers 245F/345R and 312F/412R.[24] To characterize the gene cassettes, a PCR with subsequent sequence analysis (AGOWA GmbH, Berlin, Germany) was performed on isolates containing *intI1* using primer pair L1/R1.[25] All of the above-mentioned PCRs exploring the presence of resistance determinants have been applied to the parental strains as well as to the transconjugants.

The antimicrobial susceptibility of all *E. coli* isolates was tested by the standard disc diffusion method on Mueller–Hinton Agar (Oxoid, Wesel, Germany) following the CLSI recommendations.[13] The following 21 antimicrobial agents were tested: ampicillin, amoxicillin/clavulanic acid, cefalexin, cefazolin, cefotaxime, cefovecin, cefoxitin, imipenem, penicillin, ticarcillin, enrofloxacin, marbofloxacin, amikacin, gentamicin, neomycin, doxycycline, tetracycline, chloramphenicol, fusidic acid, meropenem and sulfamethoxazole/trimethoprim.

MICs of 15 antimicrobial agents, including six β-lactams [Table S1, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)], were determined in Micronaut-S-Kleintier plates (Merlin Diagnostika GmbH, Rüsselsheim, Germany) using the broth microdilution method following the CLSI guideline M31-A3.[13] Clinical breakpoints given in the same CLSI document were used for classification as susceptible or intermediate/resistant. For substances without a given CLSI breakpoint the highest concentration tested in the layout was used to determine an isolate as resistant. *E. coli* ATCC 25922 was used as a quality control strain as recommended by the CLSI.

Conjugation experiments

Transfer of β-lactam resistance was performed by the filter mating method using *E. coli* J53 Azi^R as recipient.[26] Mating experiments were performed at 37°C and 22°C in liquid and solid (filter) media. Transconjugants were selected on trypticase soy agar, containing 100 g/mL sodium azide and 10 g/mL ceftazidime. Co-transfer of resistance determinants was explored by amplifying the respective genes in the transconjugants as described above, followed by sequence analysis, if necessary.

PFGE

To reveal a possible clonal origin of O25b-ST131 ESBL- and non-ESBL-producing *E. coli* isolates macrorestriction analysis was performed as previously described using a CHEF DRIII System (Bio-Rad, Munich, Germany).[27] PFGE profiles generated by restriction of chromosomal DNA with XbaI and NotI were compared digitally using BioNumerics software (Version 4.6; Applied Maths, St-Martens-Latem, Belgium). Cluster analysis of Dice similarity indices based on the unweighted pair group method with arithmetic mean (UPGMA) was applied to generate dendrograms depicting the relationships among PFGE profiles. Isolates were considered to belong to a group of clonally related strains if the Dice similarity index of the PFGE pattern was ≥85%.[28]

Virulence gene typing

All 16 ST131 *E. coli* isolates (ESBL and non-ESBL producers) were investigated for the presence of genes linked with extra-intestinal and intestinal pathogenicity by multiplex and single PCRs as described previously.[29–33] Genes and descriptions are given in Figure 3.

Results

O typing

Of the 177 ESBL-producing *E. coli* isolates, 10 (5.6%) were positive for molecular subgroup O25b. The presence of the O25b subgroup, determined at the molecular level, was verified for most of the ESBL-positive strains, in that they were typed as Ont (rel.25):H4 (O antigen non-typeable but related to O25) by standard agglutination tests in the German reference centre. The results of molecular typing of the *rfbO25* locus only differed in the case of the horse strain IMT18354, which was delineated to serotype O86:H51. With the exception of the human UTI strain IMT9087, which was typed as Orough:H5, the other four presumed non-ESBL ST131 strains could also be allocated to the O25b molecular subgroup.

MLST and phylogenetic grouping

All 10 phenotypically ESBL-producing *E. coli* positive for the *rfbO25b* locus were allocated to ST131 and phylogenetic group B2, as determined by MLST and structure analysis. The human reference strain DSM22664 (S. M. Soto, E. Junker, W. Barownick, A. Schroeter, R. Helmuth and B. Guerra, unpublished data) as well as five other *E. coli* strains included for comparative purposes have been previously typed as B2-ST131 strains (C. Ewers, T. Semmler and L. H. Wieler, unpublished data).

Determination of phenotypic and genotypic resistance

The phenotypical confirmatory test for production of ESBL was positive for the 10 clinical isolates, while the five comparative ST131 isolates tested negative and were thus identified as non-ESBL producers. Nine of the O25b-ST131 ESBL isolates harboured a CTX-M-15-type β-lactamase enzyme (pI 8.9) and the respective gene $bla_{\text{CTX-M-15}}$, while strain IMT12556 possessed an SHV-12-type ESBL (pI 8.2) and the corresponding bla_{SHV} gene, as determined by IEF, PCR amplification and subsequent sequence analysis. The bla_{SHV} and $bla_{\text{CTX-M-15}}$ genes were located on the respective plasmids, as determined by Southern blotting and subsequent hybridization analyses. Multireplicon and single replicon plasmids carrying IncI (IMT12556), IncFIA (IMT18342), IncFIA and IncFIB (IMT17433, IMT17530, IMT18582 and IMT19336) and IncI and FIA (IMT17898) replicons were identified in animal ESBL strains by Southern blot and hybridization analyses; none of the strains was positive for the FII replicon. Self-transferability could be demonstrated for all CTX-M-15 as well as for the SHV-12-type ESBL-harbouring plasmids. Among the genes co-transferred with the plasmids of the respective strains were $bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-12}}$, *inc/rep* genes, *aac(6′)-Ib-cr*, $bla_{\text{OXA-group-1}}$, *strA*, *strB*, *intI1* and *aadA* indicating a co-localization of these genes listed in Figure 1.

As depicted in Figure 2, the B2-O25b-ST131 ESBL-producing animal *E. coli* strains originated from five European countries, Spain, Denmark, the Netherlands, France and Germany, and were mostly isolated from UTIs (70%), while the remaining three strains were associated with WI, respiratory tract infection and eye inflammation, respectively. With respect to all ESBL-producing *E. coli* examined in this study, the number of B2-O25b-ST131 ESBL-positive strains originating from UTI in dogs accounts for 16.3% (7/43), those from WIs in dogs for 4.2% (1/24) and the only one from a horse for 2.0% (1/50).

Other resistance genes detected among the ST131 *E. coli* isolates are shown in Figure 1. The presence of genes previously associated with plasmids harbouring $bla_{\text{CTX-M-15}}$ genes, such as the $bla_{\text{OXA-1}}$ group and *acc(6′)-Ib-cr*, was determined in 10 O25b-ST131 ESBL-positive animal strains by PCR and sequence analysis. Seven isolates contained both the *acc(6′)-Ib-cr* and the $bla_{\text{OXA-1}}$ group genes, while these genes were not present in the only CTX-M-15 ESBL-producing strain isolated from a WI in a dog (IMT18582) and in the SHV-12-type ESBL-producing strain IMT12556. In addition, as illustrated in Figure 1, some strains harboured $bla_{\text{TEM-1}}$, $bla_{\text{TEM-16}}$ genes, while none of the strains was positive for the *qnrA*, *qnrB* and *qnrS* genes.

The *intI1* gene encoding class 1 integrase was detected in four out of five sulfamethoxazole/trimethoprim-resistant animal isolates, three ESBL-producing strains and one non-ESBL-producing strain. All of them amplified the class 1 integron variable region. The gene cassette arrangements detected among the *intI1*-positive animal isolates were *dfrA1-aadA1* (two isolates) and *dfr17-aadA5* (two isolates). All but one class 1 integron-carrying isolates were *sul1* negative. None of the 16 ST131 strains carried the *intI2* gene, specific for class 2 integrons.

Overall, the five non-ESBL-producing strains showed resistance to only a few antimicrobial substances, as determined by a standard disc diffusion method (Figure 1). In contrast, all ESBL-producing *E. coli* displayed different multiresistant phenotypes

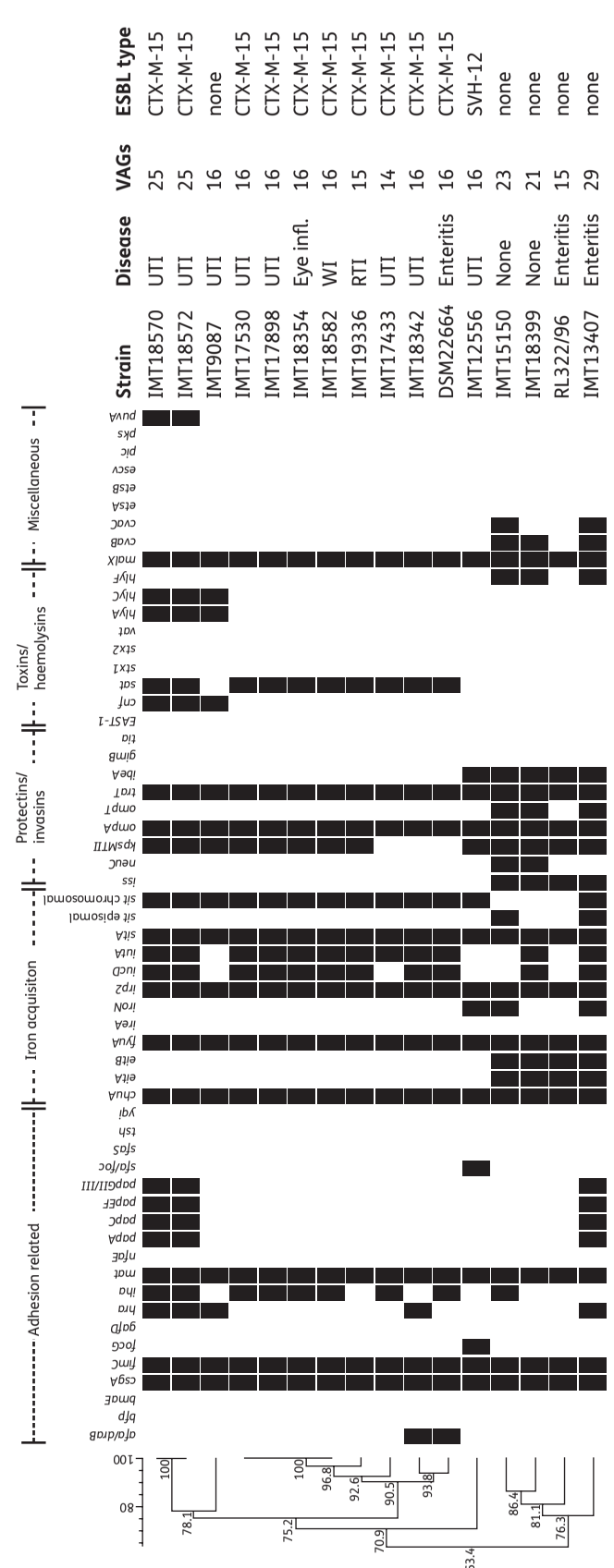
Strain designation	ESBL type	Amplicillin	Amoxicillin/clavulanic acid	Cefalexin	Cefazolin	Cefotaxime	Cefovecin	Cefoxitin	Imipenem	Penicillin	Ticarcillin	Enrofloxacin	Marbofloxacin	Amikacin	Gentamicin	Neomycin	Doxycycline	Tetracyciline	Chloramphenicol	Fusidic acid	Meropenem	Trimethoprim/sulfamethoxazole	Other resistance genes detected[a]	Presence of class I and class II integron types
IMT13407	none	R	I							R	R						R	R		R		R	*tet* (A), *sul2*, bla_{TEM-1}	*dfrA1-aadA1* (class I)
RL322/96	none									R										I			bla_{TEM-1}	none
IMT18399	none	R	R							R	R						R	R		R			*tet* (A), *sul2*, bla_{TEM-1}	none
IMT9087	none	I	I							R							R	R	R	R			*tet* (A), *tet* (B), $bla_{TEM-116}$	none
IMT15150	none									R										R			bla_{TEM-1}	none
IMT12556	SHV-12	R		R	R	R	R			R	R	R				I	R	R		R		R	*strA, strB, tet* (A), *aac(6′)-IB-cr*, bla_{TEM-1}	*dfrA1-aadA1* (class I)
IMT17433	CTX-M-15	R	I	R	R	R	R			R	R	R	R				R	R		R			*tet* (A), *strA, strB, aacC4, aac(6′)-IB-cr*, bla_{TEM-1}	none
IMT17530	CTX-M-15	R	R	R	R	R	R			R	R	R	R		R		R	R		R		R	*tet* (A), *sul1, strA, strB, aac(6′)-IB-cr*, bla_{TEM-1}, $bla_{OXA-group-1}$	*dfrA17-aadA5* (class I)
IMT17898	CTX-M-15	R	I	R	R	R	R			R	R	R	R				I	I		R			*strA, strB, aac(6′)-IB-cr*, bla_{TEM-1}, $bla_{OXA-group-1}$	none
IMT18342	CTX-M-15	R	I	R	R	R	R			R	R	R	R		R		R	R		R		R	*tet* (A), *sul2, strA, strB, aac(6′)-IB-cr*, bla_{TEM-1}, $bla_{OXA-group-1}$	*dfrA17-aadA5* (class I)
IMT18354	CTX-M-15	R	I	R	R	R	R			R	R	R	R		R		R	R	R	R		R	*tet* (A), *sul2, strA, strB, aac(6′)-IB-cr*, bla_{TEM-1}, $bla_{OXA-group-1}$	none
IMT18570	CTX-M-15	R	I	R	R	R	R			R	R	R	R		R		R	R		R			*tet* (A), *strA, strB, aac(6′)-IB-cr*, $bla_{TEM-116}$, $bla_{OXA-group-1}$	none
IMT18572	CTX-M-15	R	I	R	R	R	R			R	R	R	R		R		R	R		R			*tet* (A), *strA, strB, aac(6′)-IB-cr*, $bla_{TEM-116}$, $bla_{OXA-group-1}$	none
IMT18582	CTX-M-15	R		R	R	R	R			R	R	R	R				I	I	R	R			*strA, strB, aac(6′)-IB-cr*, bla_{TEM-1}	none
IMT19336	CTX-M-15	R	I	R	R	R	R			R	R	R	R				I	R		R			*tet* (A), *sul2, aadA, aac(6′)-IB-cr*, bla_{TEM-1}	none
DSM22664	CTX-M-15	R	R	R	R	R	R			R	R	R	R				R	R		R		R	*strA, strB, tet* (A), *sul2, aadA, aac(6′)-IB-cr*, bla_{TEM-1}, $bla_{OXA-group-1}$	*dfrA1-aadA1* (class I)

[a]*E. coli* strains were screened for genes *tet* (A), *tet* (B), *tet* (C), *sul1*, *sul2*, *sul3*, *strA*, *strB*, *qnrA*, *qnrB*, *qnrS*, *aadA*, *aacC4*, bla_{TEM}-*like*, bla_{SHV}, bla_{OXA}, and *aac(6′)-Ib* and its variant.

Figure 1. Results of agar diffusion test and screening of resistance genes not related to ESBL production in B2-O25b-ST131 *E. coli*. Black squares, resistant; grey squares, intermediate; white squares, susceptible.

Strain	Host	Disease	Serotype	O type	Country	ESBL type
IMT12556	Dog	UTI	Ont(rel.25):H4	O25b	Germany	SHV-12
IMT9087	Human	UTI	Osp:H5	-	Germany	None
RL322/96	Dog	Enteritis	Ont(rel.25):H4	O25b	Germany	None
IMT13407	Swine	Enteritis	Ont(rel.25):H4	O25b	Germany	None
IMT18399	Human	None	Ont(rel.25):H4	O25b	Germany	None
IMT15150	Chicken	None	Ont(rel.25):H4	O25b	Germany	None
IMT18570	Dog	UTI	Ont(rel.25):H4	O25b	Netherlands	CTX-M-15
IMT18572	Dog	UTI	Ont(rel.25):H4	O25b	France	CTX-M-15
IMT18582	Dog	WI	Ont(rel.25):H4	O25b	Denmark	CTX-M-15
DSM22664	Human	Enteritis	Ont(rel.25):H4	O25b	Germany	CTX-M-15
IMT17433	Dog	UTI	Ont(rel.25):H4	O25b	Germany	CTX-M-15
IMT17898	Dog	UTI	Ont(rel.25):H4	O25b	Spain	CTX-M-15
IMT19336	Dog	RTI	Ont(rel.25):H4	O25b	Germany	CTX-M-15
IMT17530	Dog	UTI	Ont(rel.25):H4	O25b	Denmark	CTX-M-15
IMT18342	Dog	UTI	Ont(rel.25):H4	O25b	Germany	CTX-M-15
IMT18354	Horse	Eye infl.	O86:H51	O25b	Germany	CTX-M-15

Figure 2. Dendrogram showing the relationship of ESBL- and non-ESBL-producing B2-O25b-ST131 *E. coli* isolates based on XbaI-generated PFGE profiles. Based on a similarity index of >75%, *E. coli* strains are separated into two main PFGE clusters (I and II). Closely related groups of strains (Dice similarity value>85%) are highlighted by grey-shaded boxes. A size marker (Lambda Ladder PFG Marker; New England Biolabs GmbH, Frankfurt a. M., Germany) with respective fragment sizes (kb) is given on top of the agarose gel. UTI, urinary tract infection; WI, wound infection; RTI, respiratory tract infection; infl., inflammation.



Strain	Disease	VAGs	ESBL type
IMT18570	UTI	25	CTX-M-15
IMT18572	UTI	25	CTX-M-15
IMT9087	UTI	16	none
IMT17530	UTI	16	CTX-M-15
IMT17898	UTI	16	CTX-M-15
IMT18354	Eye infl.	16	CTX-M-15
IMT18582	WI	16	CTX-M-15
IMT19336	RTI	15	CTX-M-15
IMT17433	UTI	14	CTX-M-15
IMT18342	UTI	16	CTX-M-15
DSM22664	Enteritis	16	CTX-M-15
IMT12556	UTI	16	SVH-12
IMT15150	None	23	none
IMT18399	None	21	none
RL322/96	Enteritis	15	none
IMT13407	Enteritis	29	none

Figure 3. Dendrogram illustrating the similarity of virulence gene profiles of B2-ST131 *E. coli* based on the presence (black boxes) and absence (white boxes) of a total of 58 virulence-associated genes. UTI, urinary tract infection; WI, wound infection; RTI, respiratory tract infection; infl., inflammation. The following VAGs were determined: *afa/draB*, afimbrial DR-binding adhesin; *bfp*, bundle-forming pili; *bmaE*, M-adhesin; *csgA*, curli fibre gene; *fimC*, type I fimbriae; *focG*, F1C fimbrial adhesin; *gafD*, G fimbriae; *hra*, heat-resistant agglutinin; *iha*, adhesin-siderophore receptor; *mat*, meningitis and temperature-dependent fimbriae; *nfaE*, non-fimbrial adhesin; *papA/C/EF/GII,III*, pyelonephritis-associated fimbriae; *sfa/foc*, central consensus regions of S fimbrial and F1C fimbrial operons; *sfaS*, S-fimbrial adhesin; *tsh*, temperature-sensitive haemagglutinin; *yqi*, adhesion-related gene; *chuA*, salmochelin receptor; *eitA/eitB*, iron uptake; *fyuA*, ferric yersinia uptake; *ireA*, iron-responsive element; *ironN*, siderophore receptor; *irp2*, iron-repressible protein; *iucD*, aerobactin; *iutA*, aerobactin receptor; *sitA/sit* chromosomal/*sit* episomal, members of iron-uptake system; *iss*, increased serum survival; *neuC*, K1 capsule synthesis; *kpsMTII*, group 2 capsule synthesis; *ompA*, outer membrane protein; *ompT*, outer membrane protease; *traT*, serum resistance associated; *ibeA*, invasion-associated; *gimB*, genetic island meningitis; *tia*, toxigenic invasion locus; *EAST-1*, heat-stable toxin; *cnf*, cytotoxic necrotizing factor; *sat*, secreted autotransporter toxin; *stx1/stx2*, shiga toxins; *vat*, vacuolating autotransporter toxin; *hlyA/hlyC/hlyF*, haemolysin A/C/F; *malX*, pathogenicity island marker; *cvaB/cvaC*, ColV operon genes; *etsA/etsB*, ColV plasmid-linked genes; *escv*, type III secretion system; *pic*, serin protease; *pks*, polyketide synthetase; *puvA*, virulence-associated factor.

not only to β-lactams but also to fluoroquinolones, aminoglycosides, tetracyclines, sulphonamides and chloramphenicol. No isolate showed resistance to carbapenems or to cefamycin/cefoxitin.

MIC testing of 11 ESBL-producing isolates, including the human reference strain DSM22664 revealed that all showed resistance to at least two non-β-lactam antimicrobials. Ten (90.9%) isolates were resistant to fluoroquinolones, 81.8% to tetracycline and 45.5% to gentamicin and sulfamethoxazole/trimethoprim, respectively (Table S1).

Strain IMT18354 originating from a case of eye inflammation in a horse showed the highest number of resistances. One single fluoroquinolone-susceptible *E. coli* isolated from a dog with UTI was also susceptible to all aminoglycosides tested, except for neomycin (intermediate), although it harboured the *aac(6′)-Ib-cr* gene variant, probably deficient in expression. The only isolate (IMT18582; positive for $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$) classified as susceptible to amoxicillin/clavulanic acid was isolated from a WI in a dog from Denmark. With the exception of strains IMT18570 and IMT18572, all strains exhibited different patterns of resistance.

PFGE analysis

Cluster analysis of macrorestriction patterns was performed on 10 ESBL-producing O25b-ST131 isolates from animals and for comparative analysis on five non-ESBL-producing ST131 animal *E. coli* isolates and human reference strain DSM22664 initially applying restriction endonuclease XbaI. Using a ≥75% similarity cut-off point, PFGE identified two main clusters of six (cluster I) and nine (cluster II) strains, respectively, while the ESBL horse strain IMT18354 was separated from all other strains with a similarity value of 66.5% (Figure 2). While cluster I contains all non-ESBL O25b-ST131 strains as well as the only strain producing an SHV-12-type extended-spectrum lactamase (IMT12556), cluster II groups all but the single horse CTX-M-15-type ESBL-producing strains together. Moreover, the human reference strain DSM22664 was allocated to cluster II, constituting a group of clonally related strains with six ESBL strains from animals at a similarity value of 87.0%. Within this group, two canine UTI strains, which originate from dogs in France and the Netherlands, exhibit 100% identical restriction patterns and are thus designated clones. In general, there is an inconsistent correspondence of the grouping of the strains and their geographical origin which is most apparent in the group of related strains of cluster II that includes seven strains from five different European countries.

The fact that the CTX-M-15 ESBL-producing horse strain was clearly 'outgrouped' from all other strains was verified by using the low-cut restriction endonuclease NotI, revealing a comparably low similarity value of 70.0% to the other restriction patterns and in principal confirming the grouping established by XbaI restriction (data not shown).

Virulence gene typing

Virulence gene profiles were determined for 11 O25b-ST131 ESBL- and 5 O25b- and Orough-ST131 non-ESBL-producing strains to get an idea about the diversity among strains of this ST and to assess the virulence potential of this group of phylogenetically related strains. Out of the 58 virulence-associated genes (VAGs) tested, the isolates contained between 14 and 29 genes each (Figure 3). Ten different VAGs were regularly present in all 16 isolates, including adhesin genes *csgA*, *fimC* and *mat*, as well as iron-related genes *chuA* and *sitA* and high-pathogenicity island (PAI) (HPI)-associated iron-acquisition genes *fyuA* and *irp2*. All ST131 strains were also positive for serum-resistance-related genes *ompA* and *traT*, and for PAI marker gene *malX*. Other VAGs, such as group II capsule synthesis gene *kpsMTII* and yersiniabactin gene *iucD* and its receptor gene *iutA* were present in at least 75% of the strains. Genes located on big plasmids, such as ColV, ColBM and others, were not present in any of the ESBL-producing isolates, while such genes, e.g. *cvaB*, *cvaC*, *eitA*, *eitB*, *iroN*, *ompT* and *hlyF*, could be irregularly detected in non-ESBLs.

Apart from the 'high PAI', also designated PAI IV_{536} (*fyuA*/*irp2*), that was observed to be present in all strains, other classical extra-intestinal PAIs were determined among the strains. PAI I_{CFT073}, harbouring genes *iha*, *papC*, *papG* allele II, *iutA* and *hlyA* was identified in clonal ESBL-producing strains IMT18570 and IMT18572. These strains, as well as another non-ESBL-producing strain (IMT13407) in addition possessed PAI II_{J96}-related genes *hra*, *papC* and *papG* allele III. SHV-12-type-positive strain IMT12556 exclusively harboured PAI III_{536}-related genes *sfa*/*foc* and *iroN* in combination.

Overall, virulence pattern similarity among the 16 isolates differed considerably, ranging from 63.4% to 100% (Figure 3). Two groups of two and four strains respectively exhibited identical VAG patterns. The first group includes the two canine CTX-M15-producing UTI strains that have already been shown to display identical PFGE patterns (Figure 2). However, this is the only exceptional finding as, in general, VAG patterns corresponded rather inconsistently with PFGE group and geographical and clinical origin of the strains. Moreover, there was no association between the virulence profile and the ESBL status of the ST131 strains.

Discussion

One key factor involved in the current expansion of ESBL-producing *E. coli* is clonal spread, as exemplified by the global dissemination of the highly virulent clone B2-O25:H4-ST131, which is thought to be responsible for the pandemic spread of the CTX-M-15 enzyme in hospital and community settings in Europe.[3] The emergence of this group of clonally related strains has been almost exclusively observed in the human population while only one very recent study demonstrated the existence of a CTX-M-15-positive B2-O25:H4-ST131 strain in a dog.[3,7,9] This multidrug-resistant extra-intestinal pathogen poses a significant public health threat not only because of its rapid spread through the population but also by its presumed continuous evolution. Our study was designed to assess the role of animals in this concerning scenario, addressing the question about how widely these globally distributed strains are disseminated among companion animals. Several transmission pathways could be envisaged if animals really constitute a possible reservoir or at least infection source.

Indeed, we identified six B2-O25b-ST131 CTX-M-15-positive animal strains exhibiting a PFGE pattern of >85% similarity to human strain DSM22664, which shows the features of the human pandemic clone. These isolates originated from five

different European countries, indicating geographically distant infection and/or transmission episodes. Two strains have been isolated from countries, namely France (IMT18572) and Spain (IMT17898), which are among the first to have experienced localized outbreaks involving specific human healthcare institutions, arguing in favour of transmission of these distinct multiresistant strains between human and animal hosts.[5,7] Two canine UTI strains from the Netherlands (IMT18570) and France (IMT18572) revealed 100% identity as assessed by PFGE analysis and substantiated by an identical virulence gene pattern. This impressively delineates a transboundary dissemination scenario, suggesting recent or ongoing transmission, which has only been observed for human isolates so far.[7]

With the permanent increase in clonally related multiresistant ESBL-producing *E. coli* in recent years the question arises to what extent does the transfer of resistance-encoding plasmids or the phylogenetic background in terms of clonal spread of the host bacterium itself contribute to this situation. Interestingly enough, the five non-ESBL ST131 strains that were included in our study to address the question of whether the ST predicts the ESBL status were all positive for the molecular subtype O25b. However, they did not reveal an ESBL phenotype, they did not possess any bla_{ESBL} genes and, finally, PFGE separated these strains from all CTX-M-15-positive ST131 strains. In a recent study, Leflon-Guibout *et al.*[34] identified non-ESBL-producing faecal ST131 *E. coli* isolates in 7% of independent healthy volunteers, all belonging to serotype O25. Two of these strains exhibited a clonal relationship with a subgroup of clinical CTX-M-15-positive ST131 strains isolated from France, Canada and Spain.[34] This marked similarity of the PFGE profiles strongly suggests not only a recent acquisition of CTX-M-15-mediating plasmids by clone ST131 but also recent divergence from a common ancestor. At 62.0% the overall similarity of PFGE profiles determined in that study corroborates the finding of Lau *et al.*[35] that ST131 isolates from the UK encompassed multiple PFGE groups at similarity levels markedly lower than 85%. We also observed CTX-M-15-type ESBL-producing strains, such as UTI strains IMT17530 and IMT18342, as well as the single horse strain IMT18354, which, according to the cut-off value defined, cannot be regarded as a member of the group of clonally related pandemic strains. Presuming a common ancestor this would suggest an enormous genomic diversification to have taken place within this lineage, most likely reflecting chromosomal rearrangements and horizontal transfer of mobile elements. Nevertheless, our data confidently show that a considerable proportion of animal O25-ST131 CTX-M-15 ESBL *E. coli* strains share highly similar PFGE patterns with the human reference strain DSM22664.

Apart from CTX-M-15-positive ST131 strains, one SHV-12-type β-lactamase-producing canine UTI strain was observed, which was allocated to PFGE cluster I of non-ESBL strains, separating it from the other animal ESBL strains. Although most of the previously published works reported ST131 strains to be associated with CTX-M-15-type enzymes, others support our finding that ESBL-producing ST131 strains are not strictly confined to this enzyme type. Likewise, six different ESBL types have been identified in a collection of eight ST131 human ESBL strains in Spain only recently, arguing in support of the existence of multiple reservoirs of such strains, from which they spread to various regions of the world acquiring plasmids carrying different ESBL-encoding genes.[8] This is consistent with the detection of an O25b:H4-ST131 CTX-M-14-producing strain in Europe that is currently regarded as a new emergent clone linked with clinical outbreaks in hospital settings in Japan and other Asian countries.[5,36] This seems to be an ongoing process as we only recently isolated an O25b:H4-ST131 strain producing CTX-M-9 from a feral urban rat.[37] Moreover our data and those of others[38,39], unlike most previously published studies, show that the CTX-M-15 allele is not regularly located on plasmids with replicon types FII-FIA or FII-FIB, but rather on IncFI plasmids, likely representing equivalent vehicles for the dissemination of bla_{ESBL} genes.

The importance of the O25-ST131 CTX-M-15 clonal group can be inferred not only from its multiresistance phenotype but also from its extra-intestinal virulence capability. Although a negative correlation between antimicrobial resistance and the expression of extra-intestinal VAGs has been reported for *E. coli* strains, this seems to be contradictory in the group of ST131 ESBL strains.[5–7,9,40] Several authors have observed a lack of important extra-intestinal virulence factors, such as P, Dr and S adhesins, Hly and CNF1 toxins and K1 invasive antigen. Conversely to that, others identified a substantial virulence potential of CTX-M-15-positive isolates of clone O25:H4-ST131, deduced from either the presence of classical extra-intestinal VAGs or their capability to produce biofilms and to be highly virulent in a mouse lethality assay.[6,7] In accordance with these studies we observed high numbers of VAGs and a considerable diversity of virulence determinants carried by animal ST131 isolates (Figure 3). As expected, no specific pattern or gene was identified, allowing for differentiation of different ESBL types (CTX-M-15 and SHV-12) or of ESBL from non-ESBL ST131 strains in general. The lack of plasmid-related genes in our ESBL-positive strains could be due to identical incompatibility classes of multidrug-resistant and well-known virulence-associated plasmids, including IncFI replicon type plasmid ColV.[31] In contrast, non-ESBL-producing ST131 animal strains harboured different genes known to be located on large plasmids associated with extra-intestinal virulence, such as *eitA*, *eitB*, the episomal *sit* variant, *iss*, *ompT*, *hlyF* and ColV operon genes *cvaB* and *cvaC*. In contrast to others, we identified not only the HPI but also other classical extra-intestinal PAIs among the ST131 strains, irrespective of their ESBL status. These data reflect what has long been known as the genome plasticity of the group of extra-intestinal pathogenic *E. coli* and obviously does not spare ST131, whether a strain belonging to the ST produces an ESBL or not.[41] Thus, these animal ST131 ESBL-producing strains might pose the double threat of multidrug resistance and substantial extra-intestinal virulence capability, which has already been proposed for the respective strains in human medicine.[7]

Another worrying fact is that ESBL producers are commonly resistant not only to β-lactams but also to fluoroquinolones, aminoglycosides and sulfamethoxazole/trimethoprim, which further contributes to the selection and persistence of these strains in both clinical and community settings.[42] The increase in the proportion of ESBL-producing isolates resistant to fluoroquinolones has apparently occurred in parallel with the increase in plasmid-mediated resistance mechanisms including Qnr proteins (*qnrA*, *qnrB* or *qnrS*) or acetylases that can affect the action of certain fluoroquinolones [*aac(6′)-Ib-cr*].[21,43] All animal ESBL-producing isolates identified in our study, including that

expressing an SHV-12-type ESBL, harboured the aminoglycoside acetyltransferase variant gene *aac(6′)-Ib-cr*. This gene variant has been reported to be confined to *E. coli* ST131 and has therefore been linked mainly to CTX-M-15-positive isolates in different surveys. However, our data also indicate its presence in an ST131 SHV-12-type ESBL-producing *E. coli*, suggesting a broader distribution, as originally presumed.[3,9,43]

Our findings demonstrate that the group of clonally related human B2-O25:H4-ST131 CTX-M-15-type ESBL-producing strains seems to be likewise present in companion animals belonging to different species and originating from different European countries. This highlights the possibility of intra-species transmission of the pandemic B2-O25:H4-ST131 CTX-M-15-type ESBL-producing clonal group from human to animals and vice versa. The importation of ESBL-producing bacterial strains into the community through animals, be it by pets or, and this should likewise be explored in the future, eventually also by farm animals, has to be considered a major factor expediting the wide dissemination of antibiotic resistance among countries and their spread to humans. The data place emphasis on the urgent need for national and supra-national efforts to implement programmes for the surveillance and epidemiologic typing of multiresistant bacteria in an interdisciplinary way considering not only the human but also the veterinary medical field as well as environmental health components. This would hopefully benefit the development of intervention and prevention strategies to control the spread of such multiresistant clonally related groups of strains in clinical settings as well as in the community and the environment.

Acknowledgements

We would like to thank F. Aarestrup, H. Hasman, L. Martínez, A. Carattoli and S. M. Soto for providing ESBL-producing control strains and Y. Pfeiffer for providing recipient strain *E. coli* J53 Azi^R. We especially thank W. Barownick and B. Lauzat for their excellent technical assistance.

Funding

This work was supported by the Federal Ministry of Education and Research Network Zoonosis (FBI-Zoo, grant no. 01KI07120), the Federal Institute for Risk Assessment (BfR46-001) and the Med-Ved-Net (EU funded Network of Excellence for the Prevention and Control of Zoonoses).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Canton R, Coque TM. The CTX-M β-lactamase pandemic. *Curr Opin Microbiol* 2006; **9**: 466–75.

2 Carattoli A. Animal reservoirs for extended spectrum β-lactamase producers. *Clin Microbiol Infect* 2008; **14** Suppl 1: 117–23.

3 Coque TM, Baquero F, Cantón R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008; **13**: 1–11.

4 Livermore DM, Canton R, Gniadkowski M *et al*. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; **59**: 165–74.

5 Blanco M, Alonso MP, Nicolas-Chanoine MH *et al*. Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2009; **63**: 1135–41.

6 Clermont O, Lavollay M, Vimont S *et al*. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. *J Antimicrob Chemother* 2008; **61**: 1024–8.

7 Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al*. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273–81.

8 Oteo J, Diestra K, Juan C *et al*. Extended-spectrum β-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. *Int J Antimicrob Agents* 2009; **34**: 173–6.

9 Pomba C, da Fonseca JD, Baptista BC *et al*. Detection of the pandemic O25-ST131 human virulent *Escherichia coli* CTX-M-15-producing clone harboring the *qnrB2* and *aac(6′)-Ib-cr* genes in a dog. *Antimicrob Agents Chemother* 2009; **53**: 327–8.

10 Johnson JR, Miller S, Johnston B *et al*. Sharing of *Escherichia coli* sequence type ST131 and other multidrug-resistant and urovirulent *E. coli* strains among dogs and cats within a household. *J Clin Microbiol* 2009; **47**: 3721–5.

11 Johnson JR, Menard M, Johnston B *et al*. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. *Antimicrob Agents Chemother* 2009; **53**: 2733–9.

12 Wirth T, Falush D, Lan R *et al*. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 2006; **60**: 1136–51.

13 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Third Edition: Approved Standard M31-A3*. CLSI, Wayne, PA, USA, 2008.

14 Rodriguez I, Barownick W, Helmuth R *et al*. Extended-spectrum β-lactamases and AmpC β-lactamases in ceftiofur-resistant *Salmonella enterica* isolates from food and livestock obtained in Germany during 2003–07. *J Antimicrob Chemother* 2009; **64**: 301–9.

15 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

16 Guerra B, Junker E, Miko A *et al*. Characterization and localization of drug resistance determinants in multidrug-resistant, integron-carrying *Salmonella enterica* serotype Typhimurium strains. *Microb Drug Resist* 2004; **10**: 83–91.

17 Bertrand S, Weill FX, Cloeckaert A *et al*. Clonal emergence of extended-spectrum β-lactamase (CTX-M-2)-producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). *J Clin Microbiol* 2006; **44**: 2897–903.

18 Bert F, Branger C, Lambert-Zechovsky N. Identification of PSE and OXA β-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. *J Antimicrob Chemother* 2002; **50**: 11–8.

19 Boerlin P, Travis R, Gyles CL *et al*. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Appl Environ Microbiol* 2005; **71**: 6753–61.

20 Park CH, Robicsek A, Jacoby GA *et al*. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother* 2006; **50**: 3953–5.

21 Robicsek A, Strahilevitz J, Sahm DF *et al*. *qnr* prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. *Antimicrob Agents Chemother* 2006; **50**: 2872–4.

22 Grimm V, Ezaki S, Susa M *et al*. Use of DNA microarrays for rapid genotyping of TEM β-lactamases that confer resistance. *J Clin Microbiol* 2004; **42**: 3766–74.

23 Jouini A, Vinué L, Slama KB *et al*. Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J Antimicrob Chemother* 2007; **60**: 1137–41

24 Skurnik D, Le Menac'h A, Zurakowski D *et al*. Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. *Antimicrob Agents Chemother* 2005; **49**: 3062–5.

25 Maguire AJ, Brown DF, Gray JJ *et al*. Rapid screening technique for class 1 integrons in Enterobacteriaceae and nonfermenting Gram-negative bacteria and its use in molecular epidemiology. *Antimicrob Agents Chemother* 2001; **45**: 1022–9.

26 Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**: 797–9.

27 Ewers C, Janssen T, Kiessling S *et al*. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. *Vet Microbiol* 2004; **104**: 91–101.

28 Carrico JA, Pinto FR, Simas C *et al*. Assessment of band-based similarity coefficients for automatic type and subtype classification of microbial isolates analyzed by pulsed-field gel electrophoresis. *J Clin Microbiol* 2005; **43**: 5483–90.

29 Muller D, Hagedorn P, Brast S *et al*. Rapid identification and differentiation of clinical isolates of enteropathogenic *Escherichia coli* (EPEC), atypical EPEC, and Shiga toxin-producing *Escherichia coli* by a one-step multiplex PCR method. *J Clin Microbiol* 2006; **44**: 2626–9.

30 Rodriguez-Siek KE, Giddings CW, Doetkott C *et al*. Characterizing the APEC pathotype. *Vet Res* 2005; **36**: 241–56.

31 Johnson TJ, Siek KE, Johnson SJ *et al*. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. *J Bacteriol* 2006; **188**: 745–58.

32 Bingen E, Picard B, Brahimi N *et al*. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. *J Infect Dis* 1998; **177**: 642–50.

33 Ewers C, Antao EM, Diehl I *et al*. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. *Appl Environ Microbiol* 2009; **75**: 184–92.

34 Leflon-Guibout V, Blanco J, Amaqdouf K *et al*. Absence of CTX-M enzymes but high prevalence of clones, including clone ST131, among fecal *Escherichia coli* isolates from healthy subjects living in the area of Paris, France. *J Clin Microbiol* 2008; **46**: 3900–5.

35 Lau SH, Kaufmann ME, Livermore DM *et al*. UK epidemic *Escherichia coli* strains A–E, with CTX-M-15 β-lactamase, all belong to the international O25:H4-ST131 clone. *J Antimicrob Chemother* 2008; **62**: 1241–4.

36 Suzuki S, Shibata N, Yamane K *et al*. Change in the prevalence of extended-spectrum-β-lactamase-producing *Escherichia coli* in Japan by clonal spread. *J Antimicrob Chemother* 2009; **63**: 72–9.

37 Guenther S, Grobbel M, Beutlich J *et al*. Detection of pandemic B2-O25-ST131 *Escherichia coli* harbouring the CTX-M-9 extended-spectrum β-lactamase type in a feral urban brown rat (*Rattus norvegicus*). *J Antimicrob Chemother* 2010; **65**: 582–4.

38 Gonullu N, Aktas Z, Kayacan CB *et al*. Dissemination of CTX-M-15 β-lactamase genes carried on Inc FI and FII plasmids among clinical isolates of *Escherichia coli* in a university hospital in Istanbul, Turkey. *J Clin Microbiol* 2008; **46**: 1110–2.

39 Mshana SE, Imirzalioglu C, Hossain H *et al*. Conjugative IncFI plasmids carrying CTX-M-15 among *Escherichia coli* ESBL producing isolates at a University hospital in Germany. *BMC Infect Dis* 2009; **9**: 97.

40 Branger C, Zamfir O, Geoffroy S *et al*. Genetic background of *Escherichia coli* and extended-spectrum β-lactamase type. *Emerg Infect Dis* 2005; **11**: 54–61.

41 Dobrindt U. (Patho-)genomics of *Escherichia coli*. *Int J Med Microbiol* 2005; **295**: 357–71.

42 Cantón R, Novais A, Valverde A *et al*. Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2008; **14**: 144–54.

43 Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother* 2005; **56**: 463–9.