Characterization of bla_{CTX-M} IncFII plasmids and clones of Escherichia coli from pets in France

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Objectives: To characterize bla_{CTX-M} IncFII plasmids and clones of Escherichia coli from cats and dogs and to compare them with bla_{CTX-M} IncFII plasmids reported in humans.

Methods: From December 2006 to April 2010, 518 E. coli isolates from clinical infections in cats and dogs were screened for extended-spectrum β-lactamase (ESBL) production. Antimicrobial susceptibility was performed by disc diffusion and resistance genes were identified by PCR and sequencing. Plasmids were characterized using PCR-based replicon typing and sub-typing schemes, restriction fragment length polymorphism analysis, S1-PFGE and Southern hybridization. Isolates were characterized by PFGE, phylogenetic grouping, O25b typing and multilocus sequence typing.

Results: Nineteen E. coli isolates (3.7%) produced ESBLs, of which 14 (74%) carried bla_{CTX-M} IncFII plasmids. The bla_{CTX-M-15} gene was predominant and located on F31:A4:B1, F36:A4:B1 or F36:A1:B20 plasmids, abundantly reported in humans. The bla_{CTX-M-15} F22:A1:B20 or F2:A2:B20 plasmids were also found. Different sequence types (STs) were identified, such as ST10, ST410, ST359, ST617 and ST224. Only one E. coli isolate belonged to the ST131 E. coli clone and carried a bla_{CTX-M-14} F2:A2:B20 plasmid.

Conclusions: This is the first known extensive study on ESBL-producing E. coli isolates from pets in France. The ST131 clone was rare. However, the predominance of human-like bla_{CTX-M-15} IncFII plasmids suggests exchanges of these plasmids with the human reservoir.

Keywords: CTX-M, IncF, veterinary, E. coli

Introduction

Extended-spectrum β-lactamases (ESBLs) confer resistance to β-lactams in Gram-negative bacteria. In both humans and animals, ESBLs of the CTX-M type are increasingly recognized, and animals have been identified as potential sources of ESBL-producing bacteria in humans.1 In food animals, numerous ESBL prevalence studies have been set up at farms or slaughterhouses as consequences of national monitoring programmes. Contrarily, ESBL prevalence in pets relies on dedicated studies, which do not generally refer to nationwide surveys. Therefore, the prevalence of ESBL producers in pets is still poorly known at a population level, in particular in clinical isolates.2–6

The role of animals in the ESBL burden in humans (and vice versa) is complex. On the one hand, ESBL genes have been shown to spread efficiently together with specific clones, such as the sequence type (ST) 131 clone of Escherichia coli in humans.7 Of note, this clone has also been found in pets, possibly reflecting transmission from owners.8,9 On the other hand, plasmids seem to play an even greater role than clonal dissemination in the spread of those genes.10 and sharing of reservoirs of bla_{CTX-M} plasmids between humans and animals has been reported.11

In pets, bla_{CTX-M}, IncFII plasmids were recently reported in Asia and the USA.3,5,12 Here, we describe the first data on the prevalence of ESBL-producing E. coli in diseased cats and dogs in France, and we specifically characterize the bla_{CTX-M} IncFII plasmids that were the major contributors to ESBL prevalence in this animal reservoir over the period of the survey.

Materials and methods

Bacterial isolates and antimicrobial susceptibility testing

Between December 2006 and April 2010, 518 E. coli clinical isolates from cats (n=104) and dogs (n=414) throughout France were collected through the national surveillance network of antimicrobial resistance in animal pathogens (Resopath; http://www.resopath.anses.fr). Antimicrobial susceptibility testing was performed by disc diffusion according to the guidelines of the Antibiogram Committee of the French Society for
Microbiology (http://www.sfm-microbiologie.fr). ESBL production was confirmed by double-disc synergy. *E. coli* ATCC 25922 was used as the quality control.

**Molecular characterization and transferability of ESBL genes**

The bla_{CTX-M} genes were detected using a CTX-M group-specific multiplex PCR. For the CTX-M-1 group, an additional PCR was performed using external primers (ISEcp1L1, 5'-CAGGTGTATGACGTCG; P2D, 5'-CAGCCGTTTGTCCGTCT TAAG). The bla_{CTX-M}, bla_Amp and bla_OXA genes were screened by PCR and ampli- cons were sequenced. Transconjugants were selected on Mueller–Hinton with sodium azide (500 mg/L) and cefotaxime (4 mg/L). When necessary, transformations were performed using electrocompetent *E. coli* TOP10 as recipient and transconjugants were selected on cefotaxime-containing plates (4 mg/L). The presence of ESBL genes in the transconjugants/transformants was confirmed by PCR.

**Characterization of bla_{CTX-M} plasmids**

Plasmids were recip typed in recipient strains using the PCR-based replicon typing (PBRT) scheme, and plasmid sizes were determined using S1-treated genomic DNA followed by PFGE (S1-PFGE). Southern blots were performed on S1-PFGE with the blaCTX-M gene and IncF-specific digoxigenine (DIG)-labelled probes (Roche Applied Science, Mannheim, Germany). IncF plasmids were subjected to replicon sequence typing to de- termine the FAB formula.13 Restriction fragment length polymorphism (RFLP) analysis was performed on PstI-digested plasmid DNA from transconjugants/transformants, and Southern blots were performed on RFLP gels with a bla_{CTX-M} probe.

**PFGE, phylogrouping, screening of the ST131 clone and multilocus sequence typing (MLST)**

PFGE was performed using the restriction enzyme BlnI. Phylogenetic group- ing of the *E. coli* isolates was performed as recently published.14 The ST131 clone was searched as described previously,15 with a human ST131-positive control (courtesy of Professor Nicolas-Chanoine, Paris, France). MLST was carried out according to the protocol described on the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli).

**Results and discussion**

We found that 3.7% (19/518) of clinical *E. coli* isolates from pets in France produced ESBLs. This is similar to what has been described in other countries worldwide, except for China, which presents a higher rate.16 Of note, this ESBL prevalence refers to the pet community and does not reflect the situation in intensive care units, which has been reported occasionally.17 To date, ESBL-producing Enterobacteriaceae are still less reported in pets than in food animals. This may result from difficulties in setting up appropriate epidemiological designs for this animal population, together with the limited number of antimicrobial resistance monitoring

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Year of isolation</th>
<th>Animal</th>
<th>District</th>
<th>Disease</th>
<th>Co-resistances</th>
<th>Phylogroup</th>
<th>CTX-M enzyme</th>
<th>FAB formula</th>
<th>CTX-M plasmid size (kb)</th>
<th>ST</th>
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*CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; TET, tetracycline; TMP, trimethoprim; TOB, tobramycin; SUL, sulfonamides. Resistances in bold were also found in transconjugants/transformants.

*FAB, F1:F1A:F1B.*
programmes that include pets. In France, this issue is being addressed through the long-term Resapath network set up in 1982 (http://www.resapath.anses.fr), which now collects more than 25,000 clinical animal isolates per year. In 2006, the Resapath network extended its nationwide survey to pets, which accounted for 20.2% of all antibiograms performed in 2011.

Of the 19 ESBL-producing *E. coli* isolates, 5 carried the same bla<sub>CTX-M-1</sub> IncI1/ST3 plasmid. Here, we characterized in detail the 14 remaining *E. coli* isolates, which were recovered from unrelated animals and various infections (Table 1). All 14 *E. coli* isolates produced CTX-M enzymes. Most ESBLs were of the CTX-M-1 group (12/14 isolates), with a predominance of CTX-M-15 (7/14). CTX-M-3 and CTX-M-32 were produced once, and two isolates produced CTX-M-14. The narrow-spectrum β-lactamases TEM-1 and OXA-1 were variably found (data not shown), while the SHV enzyme was never detected. Except for 24424, all isolates had co-resistances to non-β-lactam antibiotics. Resistances to nalidixic acid (86%, 12/14), ciprofloxacin (79%, 11/14) and tetracyclines (71%, 10/14) were frequent (Table 1). Contrarily, all isolates were susceptible to amikacin, florfenicol and carbapenems.

The ESBL phenotype was transferred by conjugation for 11/14 isolates. Transformation was achieved for isolates 19332, 24417 and 24430. All 14 bla<sub>CTX-M</sub> genes were located on plasmids of the IncF incompatibility group, with sizes ranging from 48 to 190 kb (Table 1). Various alleles were observed for each FII (F2, F13, F18, F24, F31 and F36), FIA (A-, A1, A2 and A4) and FIB (B-, B1, B20 and B24) replicon. However, identical combinations (F31:A4:B1, F36:A-B-, F36:A4:B1 and F36:A1:B20) were found in different isolates (Table 1), and three of them (F31:A4:B1, F36:A4:B1 and F36:A1:B20) carried the bla<sub>CTX-M-15</sub> gene. Identical FAB formulae did not necessarily correlate with identical RFLPs and hybridization profiles. For instance, two F36:A-B- plasmids carried different bla<sub>CTX-M</sub> genes and displayed distinct RFLPs and hybridization profiles (Figure 1a). In contrast, two F36:A4:B1 and F31:A4:B1 plasmids displayed similar RFLPs and identical hybridization profiles (Figure 1b). Identical hybridization profiles were also observed between the bla<sub>CTX-M-15</sub> F36:A4:B1 and bla<sub>CTX-M-15</sub> F36:A1:B20 plasmids (Figure 1b).

The 14 *E. coli* isolates displayed 12 BlnI PFGE patterns (Figure S1, available as Supplementary data at JAC Online). Most isolates (8/14, 57%) belonged to phylogroup A, followed by phylogroups B2 (2/14, 14%) and B1 (4/14, 28%) (Table 1). The same clone belonging to ST410 was recovered from a dog and a cat (24660/24686) originating from distinct geographical areas. The ST131 PCR was negative for all isolates except 24768, which was subsequently confirmed as ST131 by MLST. Clonal complex (CC) 10 was identified in four isolates (one ST10 and three ST617), while other STs were found twice (ST410 and ST361). Interestingly, a bla<sub>CTX-M-15</sub> F36:A4:B1 plasmid was identified on two different *E. coli* backgrounds (ST410 and ST617) and RFLP experiments showed similar but non-identical patterns, suggesting micro-evolution of plasmids on different genetic backgrounds. However,

![Figure 1. RFLP analysis with Southern blot hybridization. (a) Transconjugant (TC)/transformant (TF)-*E. coli* plasmid DNA digested with PstI. (b) Hybridization with a CTX-M probe. Lane 1, TC-24660; lane 2, TC-24686; lane 3, TC-22591; lane 4, TC-24433; lane 5, TC-24655; lane 6, TC-22611; lane 7, TC-23289; lane 8, TF-24417; lane 9, TC-24424; lane 10, TC-24768; lane 11, TC-25165; lane 12, TF-19332; lane 13, TC-24414; lane 14, TF-24430.](2799)
there was no particular association between certain plasmids and E. coli clones. ESBL plasmids and clones in pets were not always reported concomitantly in the same studies. Thus, the contribution of plasmid transfer versus clonal spread in this reservoir is not well understood. The pandemic human CTX-M-15-producing ST131 E. coli clone was reported to emerge in dogs, suggesting human-to-animal transfer. Here, only one ST131 isolate was found, which carried a blaCTX-M-15 gene. The limited prevalence of ST131 in pets in France is coherent with data from the Netherlands, Switzerland and Korea and may indicate that ST131 contributes poorly to the epidemiology of ESBL in pets. Besides, we identified STs already reported in pets, such as ST10, ST410 (belonging to CC23), ST359, ST617 and ST224.

Among the 19 ESBL E. coli identified in our collection, 74% carried blaCTX-M IncFII plasmids. Interestingly, the blaCTX-M-15 gene, which is highly prevalent in humans, was also predominant in pets. Such an overrepresentation of the blaCTX-M-15 gene was similarly reported in the USA, where this gene accounted for 78% of ESBL producers in pets. Moreover, the blaCTX-M-15 gene was located on IncFII plasmids abundantly reported in humans, such as the F31:A4:B1, F36:A4:B1 and F36:A1:B20 plasmids. In particular, the F31:A4:B1 plasmid is considered one of the five major groups of blaCTX-M-15 IncFII plasmids in E. coli isolates. Of note, the F31 and F36 alleles differ from each other by a single point mutation. Other plasmids found here, such as F22:A1:B20 and F2:A2:B20, have also been found to carry the blaCTX-M-15 gene in human E. coli isolates. In another study, in northern Kenya, blaCTX-M-15 IncFII plasmids were predominant in dogs and humans living in close contact. Also, in Asia, blaCTX-M-9 or blaCTX-M-14 IncFII plasmids are predominant in dogs (Korea and China, respectively), which correlates with the dominant CTX-M epidemiology in humans in those countries.

In conclusion, we provide the first known data on the prevalence of ESBL E. coli from a large collection of clinical isolates from cats and dogs in France. Most ESBL producers carried blaCTX-M IncFII plasmids, in particular blaCTX-M-15 IncFII plasmids, which are widely recognized in humans. This study reinforces the existence of common reservoirs and potential transmission routes of ESBL plasmids between humans and pets.

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Transparency declarations
None to declare.


