Non-ST131 Escherichia coli from cattle harbouring human-like bla_{CTX-M-15}-carrying plasmids

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Objectives: To characterize bla_{CTX-M-15}-carrying plasmids and lineages of nine strains of Escherichia coli from cattle.

Methods: Plasmid DNA was analysed using PCR-based replicon typing and plasmid sub-typing schemes, restriction fragment length polymorphism, S1 nuclease-PFGE and Southern hybridization. Strains were characterized by PFGE, multilocus sequence typing, phylogenetic grouping and B2-O25b:H4-ST131 (where ST stands for sequence type) clone screening. Susceptibilities to antimicrobials were determined by agar diffusion and resistance genes were characterized by PCR and sequencing.

Results: The bla_{CTX-M-15} gene was found on F31:A4:B1/IncFII and F2:A–:B–/IncFII plasmids, which have been reported abundantly in humans. On F31:A4:B1/IncFII plasmids, the bla_{CTX-M-15} gene was associated with the bla_{TEM-1}, bla_{OXA-1} and aac(6′)-Ib-cr resistance genes. The bla_{CTX-M-15} gene was also found on IncI1 plasmids of the CC31 clonal complex, recently identified in the human epidemic and virulent E. coli clone O104:H4. None of the cattle isolates belonged to the human and widespread clone B2-O25b:H4/ST131, but were mostly of new STs and of the phylogenetic groups A (n = 4), B1 (n = 3) or D (n = 2). The E. coli isolates harbouring the bla_{CTX-M-15}-carrying plasmids were genetically diverse, and were recovered from different geographical locations and farms and at different times.

Conclusions: This study demonstrates that bla_{CTX-M-15}-carrying plasmids from cattle-derived non-ST131 E. coli isolates were highly similar to those found in ST131 E. coli isolates commonly reported in humans. It also exemplifies the key role of plasmids versus clonal dissemination in the spread of the bla_{CTX-M-15} gene among cattle, and possibly between E. coli isolates detected in humans and cattle.

Keywords: E. coli, extended-spectrum β-lactamases, ESBLs

Introduction

Extended-spectrum β-lactamases (ESBLs) are widespread mechanisms of resistance in Gram-negative bacteria and ESBL genes, such as bla_{CTX-M} genes, are known to spread among different lineages of bacteria or in association with specific clones. In this respect, plasmids play a key role in the horizontal transfer of ESBL genes and molecular methods for typing ESBL-carrying plasmids contribute to our understanding of ESBL epidemiology. On the other hand, the pandemic dissemination of the bla_{CTX-M-15} gene has been attributed to the expansion of the B2-O25b:H4-ST131 (where ST stands for sequence type) clone of Escherichia coli. Interestingly, this clone has also been involved in the dissemination of the bla_{CTX-M-15} gene among companion animals in Europe. However, this gene has rarely been identified in food-producing animals, whereas the bla_{CTX-M-1} gene has been reported frequently. The aim of this work was first to evaluate whether CTX-M-15-producing E. coli from cattle might belong to the B2-O25b:H4-ST131 clonal group. The second aim was to characterize the lineage of these isolates and the features of the bla_{CTX-M-15}-carrying plasmids.

Materials and methods

Bacterial isolates, susceptibility testing and ESBL production

A total of 77 non-duplicate E. coli clinical isolates were collected from faeces of French cattle between 2007 and 2009, and were classified as...
resistant to cefotaxime according to CLSI standards. Specimens were collected from 10 geographical areas (districts) in France through the RESAPATH network, which carries out surveillance of antimicrobial resistance in animal infections in France (www.resapath.anses.fr). Isolates were identified using colony morphology and API20E tests, and susceptibility to 32 β-lactam and non-β-lactam antimicrobials was tested by disc diffusion. The E. coli ATCC 25922 strain was used as a quality control strain. ESBL production was determined by a double-disc synergy test and the MICs of cefotaxime, cefoxitin and ceftazidime were determined by Etest (AB bioMérieux, Solna, Sweden).

Characterization of resistance genes

The bla<sub>CTX-M</sub> 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 (5′-CAGCTTTTAGCTCG; P2D, 5′-CACGCTTGGCCTTTGGCGTCTAAG). The bla<sub>TEM</sub>, bla<sub>SHV</sub>, bla<sub>CTX-M</sub>, bla<sub>CTX-M</sub>, aac(6′)-Ib-cr, qnrA, qnrB and qnrS genes were screened by PCR and all amplicons were sequenced (Beckman Coulter, London, UK).

PFGE, phylogenetic grouping, screening of the B2-O25b:H4-ST131 clone and multilocus sequence typing (MLST)

PFGE was performed using the restriction enzyme XbaI. E. coli phylogenetic grouping was determined using a multiplex PCR for the chuA, yjaA and tspE4C2 genes and the B2-O25b-ST131 clone of E. coli was detected using the PCR-based assay described by Clermont et al. Human E. coli isolates of the B2-O25b:H4-ST131 clonal group were kindly provided by M.-H. Nicolas-Chanoine (Paris, France) and included as controls. MLST was carried out according to the protocol described on the E. coli MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Transferability of the bla<sub>CTX-M</sub> genes and plasmid characterization

Transferability was tested by mating assays, with E. coli K12 35 (pro met az) used as recipient. Transconjugants were selected on agar plates containing cefotaxime (10 mg/L) and sodium azide (500 mg/L). All plasmids were rep typed in both donor and recipient strains using the PCR-based replicon typing (PBRT) scheme and the sizes of plasmids were determined on 5% PFGE gels. Southern blot hybridizations were performed with the replicon typing (PBRT) scheme and the sizes of plasmids were determined using the plasmid MLST (pMLST) scheme or the replicon sequence typing (RST) method recently published. Restriction fragment length polymorphism (RFLP) was carried out on EcoRI-digested plasmid DNA from transconjugants subjected to electrophoresis on a 1% agarose gel.

Results

Isolates, susceptibility testing and gene identification

All 77 E. coli isolates were confirmed for ESBL production by the synergy test, and MIC values of ceftazidime ranged from 1 to 24 mg/L. Then, we focused the study on a subset of nine isolates showing an MIC of ceftazidime of ≥24 mg/L (Table 1). The nine isolates harboured the bla<sub>CTX-M-15</sub> gene, with an upstream-located IScp1 element. These CTX-M-15-producing E. coli isolates were from two districts in France that are >600 km apart. All isolates produced the β-lactamase TEM-1 and two isolates additionally produced OXA-1 (Table 1). All isolates were susceptible to cefoxitin and carbapenems, and resistance to

Table 1. Antimicrobial resistance and molecular characterization of the nine CTX-M-15-producing E. coli isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation District</th>
<th>β-Lactamases</th>
<th>MICs (mg/L)</th>
<th>Phylogenetic group</th>
<th>MLST</th>
<th>Additional resistances</th>
</tr>
</thead>
<tbody>
<tr>
<td>19302</td>
<td>2007</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>200771</td>
<td>2007</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>20084</td>
<td>2007</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>20104</td>
<td>2007</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>20727</td>
<td>2007</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>22001</td>
<td>2009</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>23163</td>
<td>2009</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
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<tr>
<td>24163</td>
<td>2009</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
<td>24</td>
<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
<tr>
<td>24165</td>
<td>2009</td>
<td>CTX-M-15, TEM-1, OXA-1</td>
<td>266</td>
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<td>STR, CHL, TET, NAL, ENR, OFX, CHL, TET, NAL, ENR, OFX</td>
<td></td>
</tr>
</tbody>
</table>
non-β-lactam antimicrobials varied (Table 1). None of the E. coli isolates harboured a qnr gene, but two possessed the plasmid-mediated aac(6′)-Ib-cr gene.

**Epidemiology of the strains**

PFGE patterns showed that isolates #19302 and #20071 (district #54) were from the same clone, as were isolates #20104 and #20727 (district #76) (Figure S1, available as Supplementary data at JAC Online). Each couple of strains was collected on the same farm, but were isolated at an interval of 1 month from two different calves. All other isolates were genetically unrelated (Figure S1). In district #54, the #24163 isolate (farm 1) was identified 2 years later than the #20104 and #20727 isolates (farm 2, 300 m from farm 1). In district #54 again, isolates #23161 and #24165 were also from different farms (farms 3 and 4), but these farms were ~30 km from farms 1 and 2.

**B2-025b:H4-ST131 clone and MLST**

The allele-specific PCR assay discriminating the B2-025b:H4-ST131 clone was negative for all isolates compared with the human controls (not shown). MLST analysis demonstrated mostly new ST types (Table 1), with the exception of isolate #23161, which was an ST88 strain belonging to the CC23 clonal complex. Isolates belonged to the phylogenetic groups A (n = 4), B1 (n = 3) or D (n = 2), but not to the B2 group (Table 1).

**Transferability and molecular characteristics of blaCTX-M-positive plasmids**

The two IncF and IncI1 replicons were found either together (5/9 isolates) or separately (4/9 isolates) in the donor strains (Table 1). The blaCTX-M-15-carrying plasmid was transferred by conjugation for all isolates, together with a single replicon and other resistance genes (Table 2). Southern blot analysis of S1-PFGE gels demonstrated the blaCTX-M-15 gene was carried either on an IncI1 (six isolates) or an IncF (three isolates) plasmid. Five out of the six blaCTX-M-15-IncI1 plasmids were of the CC31 clonal complex, i.e. they were of ST31 (#20104, #20727 and #23161) or ST68 (#24163 and #24165), a new ST described in this study (Table 2). By RFLP, the scaffolds of the ST31 and ST68 IncI1/CTX-M-15 plasmids were similar (Figure S2, available as Supplementary data at JAC Online). The blaCTX-M-15-IncFII plasmids were assigned to the F31:A4:B1 and the F2:A–B– formula, respectively. Analysis of the nucleotide sequence of the CopA RNA also showed different CopA sequences. Indeed, in Tc-22091 (indicated as FII-1, Table 2), CopA was 100% identical to that of the FII NR1 reference plasmid sequence (EMBL accession no. X02302), whereas in Tc-19302/Tc-20071 (indicated as FII-2, Table 2), CopA was 100% identical to the one reported by Hopkins et al.8

**Discussion**

We showed that CTX-M-15-producing E. coli recovered from bovine clinical diagnostic specimens did not belong to the B2-025b:H4-ST131 clonal group, but were mostly of new STs.
This differs from what has been observed in companion animals, where the presence of this clone might indicate transmission from owners to dogs.

On the other hand, in humans the ST131 clone proved to be highly prevalent in healthy subjects. Consequently, the association between \textit{bla} \textit{CTX-M-15} and ST131 isolates may result from the fact that the \textit{bla} \textit{CTX-M-15} gene has entered a widely disseminated human commensal organism. Similarly, in animals, efforts are required to explore which \textit{E. coli} clones are dominant in the normal flora and how they distribute between ESBL and non-ESBL carriers.

In our collection, the \textit{bla} \textit{CTX-M-15} gene was carried equally on IncI1 or IncFII plasmids, and highly related plasmids have previously been identified in human \textit{E. coli} isolates. We found the \textit{bla} \textit{CTX-M-15} gene on a 150 kb F31:A4:B1/IncFII plasmid, which is highly similar to a combination that has been reported in humans in the UK (2003) and Italy (2006). We also found the \textit{bla} \textit{CTX-M-15} gene on a 65 kb F2:A–:B–/IncFII plasmid, and \textit{bla} \textit{CTX-M-15}-carrying F2:A–:B– plasmids are considered among the most frequent \textit{bla} \textit{CTX-M-15}-carrying plasmids in the Enterobacteriaceae. Some of these R100-derivative plasmids were also characterized in detail, such as pEK516 and pC15-1a. Finally, we found the \textit{bla} \textit{CTX-M-15} gene on IncI1 plasmids of the CC31 clonal complex, as in the recently reported O104:H4 epidemic \textit{E. coli} human clone. In all, our results demonstrate a shared pool of \textit{bla} \textit{CTX-M-15} plasmids between cattle and humans and highlight the role of plasmid versus clonal dissemination in the spread of ESBL genes between animals and humans or vice versa. This hypothesis would also be compatible with exchanges between animals and humans of \textit{E. coli} isolates only transiently colonizing one or the other host.

IncI plasmids are highly prevalent in animals. Here, closely related \textit{bla} \textit{CTX-M-15}-carrying IncI plasmids were identified in four different locations, albeit in the same district. Thus, plasmids again seem to facilitate the diffusion of ESBL genes more efficiently than plasmid spread in the complexity of the farm setting. Moreover, it is interesting to note that there seem to be specific geographical ESBL/IncI1/ST combinations. Indeed, the same \textit{bla} \textit{CTX-M-15}/IncI1/ST3 plasmid was shown in France in \textit{E. coli} and Salmonella enterica in poultry in and \textit{S. enterica} in cattle, whereas \textit{bla} \textit{CTX-M-15}/IncI1/ST7 plasmids were described in \textit{E. coli} and \textit{S. enterica} isolates from poultry and humans in The Netherlands. Further work is now needed to clarify the determinants of such associations between subgroups of plasmids and animal or human sources of ESBL genes.

Acknowledgements

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References