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**Characterisation of clinical and food animal *Escherichia coli* isolates producing CTX-M-15 extended-spectrum  $\beta$ -lactamase belonging to ST410 phylogroup A <sup>★</sup>**

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## ABSTRACT

Seven phylogroup A CTX-M-15-producing *Escherichia coli* isolates recovered from clinical and meat samples were further characterised. All of them belonged to sequence type ST410. Only 2 of the 22 virulence genes investigated were detected. All isolates carried the *fimH* gene encoding type 1 fimbriae, and five isolates harboured the *iucD* gene encoding aerobactin siderophore. A group of five isolates showed 81.2% similarity by pulsed-field gel electrophoresis (PFGE), comprising three clinical isolates belonging to ONT:H9 and two food isolates belonging to O55:H9. Different *HpaI* digestion patterns were observed for plasmids, but all of them belonged to IncFIB group and harboured *bla*<sub>CTX-M-15</sub> associated with *bla*<sub>OXA-1</sub>, *bla*<sub>TEM</sub>, *tetA*, *catB3* and *aac(6')-Ib* surrounded by an identical genetic environment. These findings showed the possibility of lateral gene transfer of *bla*<sub>CTX-M-15</sub> as well as other antibiotic resistance determinants between low-virulence food and clinical isolates.

## 1. Introduction

During the last decade, CTX-M-type extended-spectrum  $\beta$ -lactamases (ESBLs) have replaced TEM- and SHV-type ESBLs worldwide as the most common cause of resistance to expanded-spectrum cephalosporins. Recently, a multiresistant clone of CTX-M-15-producing *Escherichia coli* (B2 O25b:H4/ST131) has been reported as spreading all over the world [1]. In the Health Area of the Virgen Macarena Hospital in the north of Seville (Spain), the predominant enzyme among ESBL-producing *E. coli* is CTX-M-14 [2]. However, the emergence of CTX-M-15 was noted in October 2005 and multidrug-resistant CTX-M-15-producing *E. coli* accounted for 24% of all ESBL-producers in 2007 [3]. CTX-M-15 production has mainly been found among phylogroup B2 clinical isolates, although clinical and food-producing animal isolates belonging to group A have also been detected [2,3]. The aims of this study were to analyse the genetic relatedness of phylogenetic group A isolates, their serotypes and virulence genes, and the genetic environment of their plasmid-acquired *bla*<sub>CTX-M-15</sub> genes.

## 2. Material and methods

### 2.1. Bacterial isolates

Seven CTX-M-15-producing *E. coli* isolates belonging to phylogroup A were obtained during two studies of ESBL-producers [2,3] in the Seville area. One clinical isolate was obtained in October 2005, four clinical isolates were obtained between September and December 2006, all of which were from urine samples from non-

related community patients, and two isolates were from raw turkey samples obtained in March 2007.

## 2.2. Typing of isolates

Molecular typing was carried out by *Xba*I pulsed-field gel electrophoresis (PFGE) (URL?), and multilocus sequence typing (MLST) was carried out according to the protocol specified on the *Escherichia coli* MLST website (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). A dendrogram was produced by the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on the Dice similarity coefficient with a 1.0% band position tolerance. Serotyping was performed with O and H antisera [1,4]. Virulence factors (*papEF*, *papG*, *sfa/focDE*, *afa/draBC*, *fimH*, *fimAV<sub>MT78</sub>*, *hlyA*, *cnf1*, *sat*, *cdtB*, *iucD*, *iroN*, *kpsMII*, *kpsMIII*, *neuC-K1*, *cvaC*, *iss*, *traT*, *ibeA*, *malX*, *usp* and *tsh*) were analysed by polymerase chain reaction (PCR) [4].

## 2.3. Analysis of the plasmid and genetic environment of *bla*<sub>CTX-M-15</sub>

Mating experiments were carried out with *E. coli* strain J53Azi<sup>R</sup> as well as transformation into *E. coli* DH10 by electroporation. Plasmids were extracted by the alkaline lysis method. Plasmid DNA from the transconjugants and electrotransformants was analysed using restriction of S1 nuclease, *Hpa*I restriction and PCR-based replicon typing [5].

Genes associated with the multidrug resistance region harbouring *bla*<sub>CTX-M-15</sub> were sought in transconjugants and transformants by PCR using primers previously

described for junction sequences 1–6 and resistance genes [6]. Other genes [*aac(3)-II* and *catB3-3'*] were screened using primer pairs designed for this study (*aac3IIF*, 5'-TGCCTCACTTAAAGCGATTG-3'; *aac3IIR*, 5'-CAAGCATCGGCATCTCATAC-3'; *catb3F*, 5'-ATCTGTTTCCGGACCGTG-3'; and *catb3R*, 5'-CTTTTGTCAACCAACGAGCG-3'). In addition, *HpaI*-digested plasmid DNA from transformants and transconjugants was hybridised using *bla*<sub>CTX-M-15</sub>, *aac(6')-Ib* and *aac(3)-II* probes. CTX-M-15-producing *E. coli* belonging to sequence type ST131 was used as a control strain [3].

The genetic environment of *bla*<sub>CTX-M-15</sub> was investigated by PCR and sequencing using primers for upstream insertion elements IS26 [7] and IS*Ecp1* [7] and for the putative promoter region (*bla*/PF, 5'-GCCAGTGACATCGTCCCATTGACG-3'; and PR, 5'-TGCTCTGTGGATAACTTGCAGAG-3').

### 3. Results

The seven phylogroup A CTX-M-15-producing *E. coli* isolates belonged to ST410 and generated seven different pulsotypes by PFGE clustering with 69.7% similarity (Fig. 1). Five of the seven pulsotypes showed 81.2% similarity and formed two clusters (>85% similarity) designated as I (isolates 281, 316 and 358) and II (isolates 113-1 and 113-6). The remaining two pulsotypes were designated as III (isolate 338) and IV (isolate 267). PFGE cluster I contained three clinical isolates belonging to ONT:H9, whilst PFGE cluster II grouped two food isolates belonging to O55:H9. PFGE III and IV isolates showed the serotypes ONT:HNM and O20:H9, respectively. Only 2 of the 22 virulence genes investigated were detected. All isolates carried the

*fimH* gene encoding type 1 fimbriae, and five isolates (281, 316, 113-1, 113-6 and 267) additionally harboured the *iucD* gene encoding aerobactin siderophore.

Cefotaxime resistance was transferred from two strains by conjugation and from five strains by electroporation. The size of plasmids was estimated as being between 89 kb and 119 kb, all of which belonged to the IncFIB group. Different *HpaI* digestion patterns were observed for plasmids obtained from isolates belonging to PFGE clusters I and II (Fig. 2).

The resistance determinants *bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>TEM</sub>, *tetA*, *catB3* and *aac(6')-Ib* as well as a partial sequence of transposon *Tn5403* were detected in all transconjugants and transformants by PCR, however *aac(3)-II* yielded negative result by PCR and positive result by hybridisation. Southern hybridisation analysis showed that *bla*<sub>CTX-M-15</sub> was located on two or three high molecular weight fragments and that *aac(6')-Ib* (Fig. 2) and *aac(3)-II* probes hybridised with the larger fragment.

Sequence analysis revealed an identical genetic environment upstream of the *bla*<sub>CTX-M-15</sub> gene for all four PFGE types: the transposase gene of *IS26* was located 64 bp upstream of a partially truncated *ISEcp1* (289 bp length), and all these sequences contained the putative promoter region for the *bla* gene (GenBank accession no. GU479916 for *E. coli* 113-1).

#### 4. Discussion

These results show the presence of the *bla*<sub>CTX-M-15</sub> gene in meat and human group A *E. coli* isolates from the same area located in related plasmids. The CTX-M-15

enzyme has been widely described for *E. coli* from humans, specifically in isolates belonging to phylogroup B2 ST131 [1], but less so from animals. The *bla*<sub>CTX-M-15</sub> gene has previously been detected in a clinical *E. coli* isolate from a cow [8], although contamination of retail meat by CTX-M-15-producing *E. coli* has not been reported. Whilst further studies are required to determine the relationship between infections caused by CTX-M-15 ESBL-producing group A isolates and the food chain, other *bla* genes such as CTX-M-9, CTX-M-14 and CTX-M-32 have been shown to be carried by poultry isolates with very similar PFGE profiles to those of clinical human isolates [9,10].

Dissemination of *bla*<sub>CTX-M-15</sub> among phylogroup A isolates, attributed as being most likely due to horizontal transfer between phylogroups B<sub>2</sub> and A, was recently described for a strain affecting 19 patients in a medical centre in Pittsburgh, PA [11]. A worrisome relationship between *bla*<sub>CTX-M-15</sub> and other resistance markers and insertion elements has been observed [6,12]. In this study, the *bla*<sub>CTX-M-15</sub> gene in the phylogroup A isolates was co-transferred with other resistance markers in IncF group plasmids and clustered in the same fragment, as previously described for phylogroup B2 isolates [6,13]. *IS26* was detected disrupting *ISEcp1*, as was the case for other *bla*<sub>CTX-M</sub> genes [7], and this organisation was different to that observed in the epidemic ST131/B2 CTX-M-15-producing pulsotypes [6,12,14].

In contrast to clonal group ST131, in which all isolates show the same serotype (O25b:H4), the seven ST410 isolates of the present study belonged to four serotypes, although six of the seven isolates carried the same flagellar antigen H9. A similar phenomenon has been described in clonal uropathogenic isolates of ST69

phylogroup D (serotypes O17:H18, O15:H18, O17:HNM, O25:H18, O73:H18, O77/17:H18, ONT:H18 and ONT:HNM) and ST95 phylogroup B2 (O1:H7, O2:H7 and O18:H7) [15].

Extraintestinal pathogenic *E. coli* harbour virulence genes required for successful pathogenic invasion of a human or animal host. The 22 virulence genes tested in the present study can be classified into at least five categories based on their function: adhesins (*papEF*, *papG*, *sfa/focDE*, *afa/draBC*, *fimH*, *fimAV<sub>MT78</sub>*); toxins (*hlyA*, *cnf1*, *sat*, *cdtB*); siderophores (*iucD*, *iroN*); protectins (*kpsMII*, *kpsMIII*, *neuC*, *cvaC*, *iss*, *traT*); and miscellaneous (*ibeA*, *malX*, *usp*, *tsh*). Only 2 of these 22 virulence genes were detected, namely the *fimH* gene encoding type 1 fimbriae and the *iucD* gene encoding aerobactin siderophore. The low virulence gene content detected suggests that the seven phylogroup A CTX-M-15-producing isolates characterised in this study may be opportunistic pathogens whose ability to cause disease can be limited to compromised hosts.

Limitations of this study include the small sample size of strains, as phylogroup A CTX-M-15-producing isolates are rarely found in surveys carried out in our area. Study strengths included the geographical matching and all isolates were recovered in an 18-month period. Although meat and clinical isolates belonged to the same sequence type, clonal spread and/or transmission from food to humans remains to be demonstrated. These findings underline the role played by an additional mechanism, apart from clonal spread of O25b:H4/B2 strains, of transmitting this gene in our area, i.e. dissemination within group A low-virulence strains via co-transfer within a multidrug resistance region in IncF plasmids. Further molecular analysis of

food and human isolates will help to better define the epidemiology of CTX-M-15-producing *E. coli* in our area.

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### **Competing interests**

None declared.

### **Ethical approval**

Not required.

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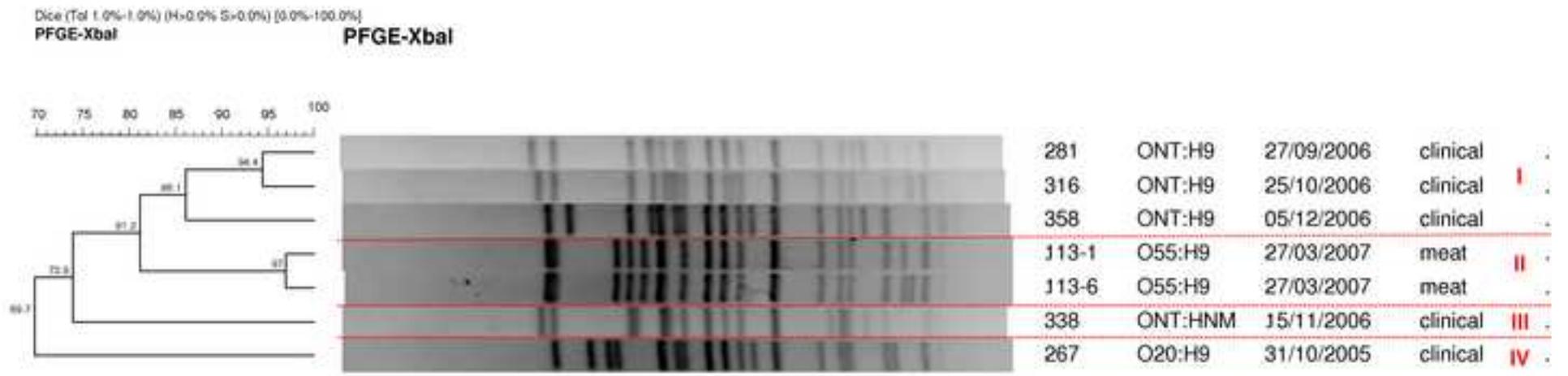
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**Fig. 1.** *Xba*I digestion pulsed-field gel electrophoresis (PFGE) dendrogram showing the relationship between the seven phylogroup A CTX-M-15-producing *Escherichia coli* isolates.

**Fig. 2.** Hybridisation of *Hpa*I-digested plasmid profiles using (B) *bla*<sub>CTX-M-15</sub> and (C) *aac(6')-Ib* probes associated with (A) *Hpa*I-digested plasmid profile of transformants (E) or transconjugants (TC). Lane M, *Hind*III-digested  $\lambda$ ; lane 1, O25:H4/ST131 B2 strain; lane 2, E281; lane 3, TC316; lane 4, E358; lane 5, E113-6; lane 6, E113-1; lane 7, E338; and lane 8, TC267.

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