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Short Communication

Chromosomal location of bla_{CTX-M} genes in clinical isolates of *Escherichia coli* from Germany, The Netherlands and the UK

I. Rodríguez[a,*], K. Thomas[a], A. Van Essen[b], A.-K. Schink[c], M. Day[d], M. Chattaway[d], G. Wu[e], D. Mevius[b,f], R. Helmuth[a], B. Guerra[a], on behalf of the SAFEFOODERA-ESBL consortium[1]

[a] *Federal Institute for Risk Assessment (BfR), Department for Biological Safety, Max-Dohrn Strasse 8–10, D-10589 Berlin, Germany*
[b] *Central Veterinary Institute (CVI) of Wageningen UR, Department of Bacteriology and TSEs, P.O. Box 65, 8200 AB Lelystad, The Netherlands*
[c] *Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), 31535 Neustadt-Mariensee, Germany*
[d] *Public Health England (PHE), 61 Colindale Avenue, London NW9 5EQ, UK*
[e] *Animal Health and Veterinary Laboratories Agency (AHVLA), Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, UK*
[f] *Department of Infectious Diseases and Immunology, Utrecht University, 3584 CL Utrecht, The Netherlands*

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ABSTRACT

This study aimed to detect and characterise clinical *Escherichia coli* isolates suspected of carrying chromosomally encoded CTX-M enzymes. *Escherichia coli* ($n = 356$) obtained in Germany, The Netherlands and the UK (2005–2009) and resistant to third-generation cephalosporins were analysed for the presence of ESBL-/AmpC-encoding genes within the European SAFEFOODERA-ESBL project. β-Lactamases and their association with IS*26* and IS*Ecp1* were investigated by PCR. Isolates were typed by phylogenetic grouping, MLST and PFGE. Plasmids were visualised by *S1* nuclease PFGE, and the location of bla_{CTX-M} genes was determined by Southern hybridisation of *Xba*I-, *S1*- and I-*Ceu*I-digested DNA. ESBL enzymes could not be located on plasmids in 17/356 isolates (4.8%). These 17 isolates, from different countries and years, were ascribed to phylogenetic groups D (9), B2 (6) and B1 (2), and to seven sequence types, with ST38 being the most frequent (7 phylogroup D isolates). Eleven isolates produced CTX-M-15. $bla_{CTX-M-15}$ genes were associated with IS*Ecp1*. The remaining isolates expressed the CTX-M group 9 β-lactamases CTX-M-14 (4), CTX-M-9 (1) and CTX-M-51 (1). bla_{CTX-M} probes hybridised with I-*Ceu*I- and/or *Xba*I-digested DNA, but not with *S1*-digested DNA, corroborating their chromosomal location. To summarise, only 4.8% of a large collection of ESBL-producing *E. coli* isolates harboured chromosomal bla_{CTX-M} genes. These isolates were of human origin and belonged predominantly to ST38 and ST131, which possibly indicates the role of these sequence types in this phenomenon. However, heterogeneity among isolates was found, suggesting that their spread is not only due to the dispersion of successful *E. coli* clones.

1. Introduction

Production of β-lactamases is the main mechanism responsible for resistance to β-lactams in Enterobacteriaceae. Extended-spectrum β-lactamases (ESBLs) are able to hydrolyse third- and fourth-generation cephalosporins and monobactams, limiting therapeutic options in serious infections caused by Enterobacteriaceae. Over the last decade, the number of ESBL-producing bacteria has increased in many different genera of Enterobacteriaceae and represents a public health threat [1].

CTX-M-type ESBLs are a complex and heterogeneous family of enzymes and may be subdivided into five major groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 groups) [2]. These enzymes have spread globally and are the most common ESBLs detected in Enterobacteriaceae, not only in hospitals but also in the community, changing the epidemiology of ESBLs. Among the different CTX-M enzymes, CTX-M-15 (belonging to CTX-M group 1) and CTX-M-14 (belonging to CTX-M group 9) are of high relevance because of their ubiquity, being detected not only in humans and animals but also in environmental samples in many different

* Corresponding author. Present address: Department of Microbiology, Hospital Universitario Ramón y Cajal, Carretera de Colmenar Viejo Km 9.100, 28034 Madrid, Spain. Tel.: +34 913 368 152; fax: +34 913 368 809.
E-mail address: irodriguezf@salud.madrid.org (I. Rodríguez).
[1] This consortium also includes: Martin J. Woodward and Nick Coldham, Animal Health and Veterinary Laboratories Agency (AHVLA), UK; Kristina Kadlec and Stefan Schwarz, Friedrich-Loeffler-Institut (FLI), Germany; John Threlfall, Neil Woodford and John Wain, Public Health England (PHE), UK; and Cindy Dierikx, Central Veterinary Institute (CVI), The Netherlands.

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countries [1,2]. The successful spread, diversification and maintenance of CTX-M enzymes is due to a combination of different factors: their association with transposable elements and integrons; the capture of these structures by conjugative plasmids; and their transfer to and maintenance in successful bacterial clones [2]. The latter are typified by *Escherichia coli* clone O25b-ST131, which is a pandemic, multiresistant and uropathogenic lineage frequently associated with the expression of CTX-M-15 and that has contributed significantly to the worldwide spread of this ESBL [3]. This clone habitually harbours the $bla_{\text{CTX-M-15}}$ gene located on plasmids belonging to the IncF family, typically IncFII or multireplicon FII, FIA and FIB [3].

Despite being predominantly plasmid-mediated enzymes, chromosomally located $bla_{\text{CTX-M}}$ genes have also been described [4–6]. In this case, the chromosomal location does not enhance the spread of the gene but does assist its stabilisation and maintenance in the bacterium. The objective of this study was to seek and characterise clinical *E. coli* isolates suspected of expressing chromosomally encoded CTX-M enzymes from Germany, The Netherlands and the UK.

2. Methods

2.1. Bacterial isolates and detection of β-lactamase-encoding genes

Within the European SAFEFOODERA-ESBL project (EU ERA-Net, Ref. 08176), a total of 629 *E. coli* isolates were selected from the strain collections of the Animal Health and Veterinary Laboratories Agency (AHVLA, UK), Public Health England (PHE, UK), Central Veterinary Institute (CVI, The Netherlands), Friedrich-Loeffler-Institut (FLI, Germany) and Federal Institute for Risk Assessment (BfR, Germany). All isolates were cefotaxime-non-susceptible [minimum inhibitory concentrations above the epidemiological cut-off of ≤0.25 mg/L for *E. coli*, set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST); http://www.eucast.org]. Isolates originated from animals (n = 295), animal-derived foods (n = 59), humans (n = 274) and an unknown source (n = 1) and were isolated between 2005 and 2009. These isolates were screened for β-lactamase and other resistance genes as well as for virulence determinants using 'Amr05' microarrays (ALERE Technologies Ltd., Stirling, UK) in a previous study [7]. For the present work, a subset of 356 isolates was selected based on the relative contribution of the β-lactamase families detected, the source of the isolates and the countries involved. β-Lactamase genes detected in these isolates with the array were further analysed by PCR sequencing as previously described [8,9]. Plasmid DNA was extracted from the 356 isolates using a Midiprep Plasmid Purification Kit (QIAGEN, Hilden, Germany) and/or the Kado and Liu method [8]. Plasmids were electrotransformed into *E. coli* ElectroMAX™ competent cells (Invitrogen-Thermo Fisher Scientific, Karlsruhe, Germany) using a Gene Pulser (Bio-Rad, Munich, Germany), 0.1 cm gap length cuvettes and the parameters 12.5 kV/cm, 200 Ω and 25 μF. Transformants were selected on Luria–Bertani agar plates containing 1 mg/L cefotaxime (Oxoid, Wesel, Germany). Isolates that were repeatedly negative in transformation experiments (repeated at least five times, using new DNA obtained by different methods, changing competent cells, electroporation machine and cuvettes) were considered potentially to have chromosomally mediated ESBLs and were studied further.

The possible association of $bla_{\text{CTX-M}}$ genes with insertion sequences IS*26* and IS*Ecp1* was investigated by PCR sequencing using the CTX-M-consensus and tnpIS*26* primers [10], or ALA-3 [11] and a modified ALA4 (5′ CTATCCG**T**ACAAGGGAG 3′), respectively.

2.2. Molecular typing and mapping of bla_{CTX-M} genes

Isolates were typed using pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested DNA, as indicated in the PulseNet protocol (http://www.pulsenet-europe.org). Plasmid content was visualised by *S1* nuclease PFGE [8]. To confirm the chromosomal location of $bla_{\text{CTX-M}}$ genes, genomic DNA was also analysed by PFGE of I-*Ceu*I-digested DNA [4]. The *Xba*I, *S1* and I-*Ceu*I PFGE profiles were transferred onto nylon membranes and were then hybridised with $bla_{\text{CTX-M}}$-specific probes [8], and additionally with a 16S rDNA probe (a 7.5-kb *BamH*I *rrnB* fragment from pKK3535) in the case of I-*Ceu*I PFGE profiles. Isolates were assigned to different phylogenetic groups by a recently modified multiplex PCR [12] and were typed by multilocus sequence typing (MLST) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

Mating experiments using isolates ESBL-105, ESBL-723, ESBL-746, ESBL-788 and ESBL-868 as donors (selected to represent different clones/enzymes) were carried out as previously described [8] on liquid broth (liquid mating) and agar (filter mating) at 22 °C and 37 °C using sodium-azide-resistant *E. coli* K12 J53

Table 1
Clinical *Escherichia coli* isolates analysed in this work.

Isolate	ESBL	Other β-lactamases	MLST	Phylogenetic group	*Xba*I PFGE [a]	Plasmids (kb)	Country	Isolation date	Origin
ESBL-91	CTX-M-14	–	ST38	D	X1	145; 78	Netherlands	2009	Human
ESBL-831	CTX-M-14	–	ST38	D	X2	125	UK	2009	Human
ESBL-884	CTX-M-14	TEM-1	ST38	D	X3	135	UK	2008	Human
ESBL-788	CTX-M-14	TEM-1	ST3878[b]	B2	X4	125; 82; 37	UK	2008	Human
ESBL-105	CTX-M-9	–	ST1266	B2	X5	–	Netherlands	2009	Human
ESBL-26	CTX-M-51	–	ST38	D	X6	115	Netherlands	2009	Human
ESBL-35	CTX-M-15	TEM-1	ST156	B1	X7	170	Netherlands	2009	Human
ESBL-229	CTX-M-15	OXA-1	ST648	D	X8	110	Germany	2009	Human
ESBL-723	CTX-M-15	OXA-1	ST38	D	X9	125; 110; 85	UK	2009	Human
ESBL-725	CTX-M-15	TEM-1	ST131	B2	X10	135; 60; 53; 30	UK	2009	Human
ESBL-772	CTX-M-15	TEM-1	ST2178	B1	X11	105; 80	UK	2009	Human
ESBL-815	CTX-M-15	TEM-1	ST38	D	X12	135; 50	UK	2008	Human
ESBL-72	CTX-M-15	–	ST131	B2	X13	105	Netherlands	2009	Human
ESBL-746	CTX-M-15	OXA-1; TEM-1	ST38	D	X14	115; 55	UK	2009	Human
ESBL-787	CTX-M-15	–	ST648	D	X15	90	UK	2008	Human
ESBL-811	CTX-M-15	TEM-1	ST131	B2	X16	90	UK	2009	Human
ESBL-868	CTX-M-15	OXA-1; TEM-1	ST131	B2	X17	80	UK	2008	Human

ESBL: extended-spectrum β-lactamase; MLST: multilocus sequence typing; PFGE: pulsed-field gel electrophoresis.
[a] Owing to the high variability of *Xba*I PFGE profiles found in the isolates, the profiles were named using X followed by a consecutive number.
[b] ST3878 is a single locus variant of ST131, which shows a different *icd* allele (208).