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Emergence of clonal groups O1:HNM-D-ST59, O15:H1-D-ST393, O20:H34/HNM-D-ST354, O25b:H4-B2-ST131 and ONT:H21,42-B1-ST101 among CTX-M-14-producing *Escherichia coli* clinical isolates in Galicia, northwest Spain

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ABSTRACT

CTX-M enzymes, mainly CTX-M-14 and CTX-M-15, have emerged as the most prevalent extended-spectrum β -lactamase (ESBL) type produced by *Escherichia coli* in Spain, with successful dissemination of clonal group O25b:H4-B2-ST131 producing CTX-M-15 within the hospital and community settings. However, until now CTX-M-14-producing *E. coli* in Spain had been shown to belong to a wide variety of serotypes with no predominance of a certain clonal group. In the present study, 654 *E. coli* strains positive for ESBL production obtained between 2005 and 2008 from inpatients and outpatients of four hospitals in Galicia, northwest Spain, were analysed. The strains were characterised with regard to ESBL enzymes, serotype, virulence genes, phylogenetic group, multilocus sequence type, and pulsed-field gel electrophoresis of *Xba*I-digested DNA. As a result, the emergence of certain clonal groups of extraintestinal pathogenic *E. coli* producing CTX-M-14 has been detected in this geographic area, including O1:HNM-D-ST59, O15:H1-D-ST393/ST1394, O20:H34/HNM-D-ST354, O25b:H4-B2-ST131 and ONT:H21,42-B1-ST101. These five clonal groups showed a high virulence potential as they harboured more than eight virulence factors, which could explain their successful dissemination.

1. Introduction

CTX-M β -lactamases were first reported from Japan in 1986 [1]. During the 1990s, general dissemination and occasional outbreaks by CTX-M-producing Enterobacteriaceae occurred [2]. Since the year 2000, however, *Escherichia coli* producing CTX-M β -lactamases have emerged worldwide as an important cause of community urinary tract infections (UTIs), a phenomenon known as ‘the CTX-M pandemic’ [3]. Clonal dissemination of CTX-M-15-producing *E. coli* belonging to phylogenetic group B2 and sequence type ST131 has been reported in different countries, including Spain [4–7]. In previous studies, we have shown that the most prevalent extended-spectrum β -lactamase (ESBL) types produced by *E. coli* isolates in the Lugo health area, northwest Spain, are CTX-M, primarily CTX-M-14 and CTX-M-15, with successful dissemination of clonal group O25b:H4-B2-ST131 producing CTX-M-15 within the hospital and community [4]. However, since its first detection in Spain [8] CTX-M-14-producing *E. coli* have been shown to belong to a wide variety of serotypes, with no predominance of a certain clonal group [4,9]. In the present study, we describe the detailed characterisation of five CTX-M-14-producing clonal groups of *E. coli* that have emerged in this health region in Galicia, northwest Spain.

2. Materials and methods

2.1. Bacterial strains

The present study included 654 *E. coli* strains detected as being positive for ESBL production in the Microbiology Unit of four hospitals in Galicia, namely Complejo Hospitalario Xeral-Calde, Lugo (325 strains), Complejo Hospitalario Universitario A

Coruña (150 strains), Complejo Hospitalario de Ourense (100 strains) and Complejo Hospitalario Arquitecto Marcide-Prof. Novoa Santos, Ferrol (79 strains). The strains included in the present study (one strain per patient) were obtained from 2005 to 2008, mainly from urine samples.

2.2. Antibiotic susceptibility testing and extended-spectrum β -lactamase typing

ESBL production was screened by cephalosporin resistance and the double-disk synergy test as described by Jarlier et al. [10]. Minimal inhibitory concentrations were determined using a MicroScan WalkAway[®] automated system (Siemens, Madrid, Spain) according to the manufacturer's instructions. Resistance was interpreted based on the recommended breakpoints of the Clinical and Laboratory Standards Institute (CLSI) [11]. To determine the genotype of ESBLs, polymerase chain reaction (PCR) and sequencing was performed using the TEM, SHV, CTX-M-1 and CTX-M-9 group-specific primers as reported previously [12].

2.3. O and H typing

Determination of O and H antigens was performed using the method previously described by Guinée et al. [13] with all available O (O1 to O181) and H (H1 to H56) antisera. Isolates that did not react with O antisera were classified as non-typeable (ONT), and non-motile isolates were denoted as HNM. Additionally, the specific O25b molecular subtype was determined by PCR [14].

2.4. Phylogenetic grouping and multilocus sequence typing (MLST)

The phylogenetic group of the *E. coli* strains (A, B1, B2 and D) was determined by the multiplex PCR-based method of Clermont et al. [15]. MLST was performed as described previously by gene amplification and sequencing of the seven housekeeping genes (*adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) according to the protocol and primers specified at the *E. coli* MLST website (<http://mlst.ucc.ie/mlst/dbs/Ecoli>) [16]. Sequence types (STs) were obtained via the electronic database at the *E. coli* MLST website.

2.5. Virulence factors

The presence of virulence genes was analysed as documented elsewhere [4,17], using primers specific for genes and operons that encode extraintestinal virulence factors (VFs) characteristic of extraintestinal pathogenic *E. coli* (ExPEC). Typing of the *afa* operon was performed using the seven pairs (*afaE1*, *afaE2*, *afaE3*, *afaE5*, *afaE7*, *afaE8* and *daaE*) of primers designed by Le Bouguéneq et al. [18] as well as the primers recently designed by Blanco et al. for the new *afa* operon FM955459 [4].

2.6. Pulsed-field gel electrophoresis (PFGE)

PFGE analysis of *Xba*I-digested DNA was performed as described previously [17]. Profiles were analysed using BioNumerics fingerprinting software (Applied Maths, St-Martens-Latem, Belgium). Cluster analysis of the Dice similarity indices based on the unweighted pair group method using arithmetic averages (UPGMA) was performed to generate a dendrogram describing the relationship among PFGE profiles.

3. Results

All 654 strains were characterised with regard to serotype and ESBL type. CTX-M-14 (334 strains; 51.1%) and CTX-M-15 (119 strains; 18.2%) were the most prevalent ESBL enzymes detected. Among CTX-M-15-producing strains, 103/119 (86.6%) belonged to clonal group O25b:H4-B2-ST131, which also produced other ESBL types (CTX-M-1, CTX-M-9, CTX-M-14 and CTX-M-32) in 10 strains.

In contrast to CTX-M-15, CTX-M-14-producing strains belonged to a wide number of serotypes. Those CTX-M-14-producing strains belonging to the same serotype were further analysed. Five CTX-M-14 clonal groups were found comprising 32 strains (25 recovered from UTIs, 4 recovered from exudates and 3 recovered from sepsis). The five clonal groups involved hospital ($n = 13$) as well as community- ($n = 10$) and healthcare-associated ($n = 9$) episodes.

3.1. Serotypes, phylogroups and sequence types

In view of the results obtained by serotyping, phylogenetic typing and MLST, five clonal groups were detected among the CTX-M-14-producing *E. coli* strains: O1:HNM-D-ST59; O15:H1-D-ST393/ST1394; O20:H34/HNM-D-ST354; O25b:H4-B2-ST131; and ONT:H21,42-B1-ST101. The single nucleotide difference between ST393 and ST1394 in one of the seven sequenced loci (*purA*) was not enough to separate strain FV 10856 into a different clonal group (Fig. 1).

3.2. Virulence genotypes

The 32 CTX-M-14-producing strains were analysed for the presence of extraintestinal VFs characteristic of ExPEC (Table 1). Equal or similar VF profiles were displayed by strains belonging to the same clonal group (Fig. 1). Furthermore, the 32 CTX-M-14 producing *E. coli* strains satisfied criteria for ExPEC status according to the definition of Johnson et al. [19]. The strains belonging to clonal groups ONT:H21,42-B1-ST101 and O1:HNM-D-ST59 accumulated the highest number of VFs (≥ 8). The two *afa/draBC*-positive strains of clonal group O25b:H4-B2-ST131 showed the *afaE1* subtype.

3.3. Antimicrobial resistance

The 32 CTX-M-14-producing *E. coli* strains were further analysed for associated resistance to carbapenems, aminoglycosides, fluoroquinolones, trimethoprim/sulfamethoxazole and fosfomycin. Strains belonging to clonal group O1:HNM-D-ST59 were susceptible to these antibiotics, whereas the remaining four clonal groups showed characteristic resistance patterns (Fig. 1). The 32 CTX-M-14-producing strains were susceptible to imipenem, amikacin and fosfomycin. Interestingly, strain FV 10827 (belonging to clonal group O25b:H4-B2-ST131) carried both *bla*_{CTX-M-14} and *bla*_{CTX-M-15} sequences.

3.4. Pulsed-field gel electrophoresis profiles

PFGE profiles of the 32 CTX-M-14 producing *E. coli* strains formed five clusters (I to V) corresponding to clonal groups O15:H1-D-ST393/ST1394, ONT:H21,42-B1-ST101, O1:HNM-D-ST59, O20:H34/HNM-D-ST354 and O25b:H4-B2-ST131,

respectively (similarities of 73.2%, 93.3%, 76.5%, 79.9% and 64.7%, respectively) (Fig. 1). Whilst clusters I, III and V grouped strains (O15:H1, O1:HNM and O25b:H4) isolated in different years from more than one hospital and showing higher genetic diversity, clusters II and IV grouped strains (ONT:H21,42 and O20:H34/HNM) isolated in Complejo Hospitalario Xeral-Calde of Lugo in 2008 and 2007, respectively, and with lower genetic variability. Thus, clonal group O15:H1-D-ST393/ST1394 (cluster I) showed a subcluster of 87.6% similarity including four strains with identical virulence profile, and the remaining strains (73.2% similarity) additionally carried the *ibeA* virulence gene; clonal group ONT:H21,42-B1-ST101 (cluster II) was the most homogeneous, with five strains of identical virulence and antibiotic resistance profile (93.3% similarity); clonal group O1:HNM-D-ST59 (cluster III) showed three subclusters (similarities 87.8%, 93.8% and 81.8%, respectively) with different virulence profiles in the carriage of *fimA*_{MT78} and *papG* alleles; clonal group O20:H34/HNM-D-ST354 (cluster IV) showed two subclusters of 83.2% and 95.8% similarity, which divided motile (H34) from non-motile (HNM) strains; and finally, clonal group O25b:H4-B2-ST131 (cluster V) showed two subclusters of 72.8% (strains negative for *afa/draBC* and positive for *fimH*) and 93.8% (strains positive for *afa/draBC* and negative for *fimH*) similarity.

To compare the O25b:H4-B2-ST131 CTX-M-14-producing strains in the present study with O25b:H4-B2-ST131 strains producing other CTX-M enzymes, nine additional O25b:H4 human strains obtained from patients with extraintestinal infections were characterised [CTX-M-9 (two strains), CTX-M-15 (six strains) and CTX-M-14 (one strain)] (Fig. 2). Pulsotypes from the 14 O25b:H4-B2-ST131 strains showed a similarity of 61.1%. Strains producing the same CTX-M type, with the

same or similar virulence profile and associated resistances, clustered together in the dendrogram.

4. Discussion

In previous studies, we have shown that CTX-M enzymes, mainly CTX-M-14 and CTX-M-15, are currently the most prevalent ESBL types produced by *E. coli* isolates in the Xeral-Calde Hospital of Lugo, with 57.1% producing CTX-M-14 and 21.9% producing CTX-M-15 among 105 ESBL-producing *E. coli* isolated from January 2006 to March 2007 [4]. We also reported the successful dissemination of clone O25b:H4-ST131 producing CTX-M-15 within the hospital and community, including across national boundaries [4,6]. The results obtained in the present study including four hospitals corroborate that CTX-M-14 (51.1%) and CTX-M-15 (18.2%) are the most prevalent ESBL types in this health area. Moreover, the majority (86.6%) of CTX-M-15-producing strains belonged to clonal group O25b:H4-B2-ST131, present in the four hospitals studied, and only a reduced number of strains belonging to this clonal group produced other CTX-M types (CTX-M-1, CTX-M-9, CTX-M-14 and CTX-M-32) [4,20].

In contrast to CTX-M-15, CTX-M-14-producing *E. coli* have been shown since their first detection in Spain [8] to belong to a wide variety of serotypes, with no predominance of a particular clonal group [4,21]. In the present study, high genetic diversity among the CTX-M-14-producing *E. coli* group was again identified but, interestingly, five CTX-M-14 clonal groups were shown to have emerged with high pathogenic potential, adding to the number of VFs and associated resistances displayed. This report is the first detailed characterisation of four of these five CTX-

M-14-producing clones (O1:HNM-D-ST59, O15:H1-D-ST393/ST1394, O20:H34/HNM-D-ST354 and ONT:H21,42-B1-ST101).

4.1. Serotypes, phylogroups and sequence types

The 32 CTX-M-14-producing *E. coli* strains characterised in the present study belonged to six serotypes (O1:HNM, O15:H1, O20:H34, O20:HNM, O25b:H4 and ONT:H21,42), three phylogroups (B1, B2 and D) and six STs (ST59, ST101, ST131, ST354, ST393 and ST1394). In Spain, Oteo et al. [9] identified 34 different STs among 41 CTX-M-14-producing *E. coli* strains recovered from 11 Spanish hospitals by the first quarter of 2004, including ST393-phylogroup D, ST101-phylogroup B1 and ST131-phylogroup B2 (one strain each). Also in Spain, Valverde et al. [21] studied 61 CTX-M-14-producing *E. coli* strains isolated between the years 2000 and 2005 and identified 20 different STs among 37 strains analysed by MLST, including ST59-phylogroup D and ST393-phylogroup D (one strain each). Clonal groups ST354-D [22,23] and ST393-D [22] have also recently been reported among human strains isolated from French and Spanish patients, but associated with CMY-2-producing *E. coli* strains; these strains were not further characterised by serotyping and pathotyping.

4.2. Clonal group O1:HNM-D-ST59

Among ExPEC strains, serogroup O1 is one of the most commonly detected among avian pathogenic *E. coli* (APEC), uropathogenic *E. coli*, newborn meningitis-causing *E. coli* and septicaemic *E. coli* strains. In a recent study on human and avian ExPEC of serotype O1:HNM/H7, Mora et al. [17] found a significant association between the

presence of virulence genes *fimAv_{MT78}* and *sat* and the human ExPEC versus APEC strains. In the present study, clonal group CTX-M-14-producing O1:K1:HNM-D-ST59 appears to have successfully spread as it was detected in four different health areas (Ferrol, Lugo, Ourense and A Coruña) through different years (2005–2008). Strains of this clonal group carried not only *fimAv_{MT78}* and *sat* genes, supporting previous results [17], but also additional VFs (≥ 8), confirming the pathogenic potential of O1:K1:HNM strains.

4.3. Clonal group O15:H1-D-ST393/ST1394

From the outbreak of 1986 in London, numerous studies have reported evidence of the continued clinical importance of this serotype [24,25]. Olesen et al. [26] recently published an epidemiological analysis with the conclusion that the O15:K52:H1 clonal group has maintained a fairly stable virulence profile since its first known appearance but, in contrast, its antimicrobial resistance profile has become progressively more extensive. In accordance with their results, we found that the five O15:H1 CTX-M-14-producing strains included in the present study belonged to phylogroup D, all but one was ST393, and all exhibited a characteristic profile of virulence genes (*fimH*, *fimAv_{MT78}*, *papGII*, *sat*, *iucD* and *kpsM II-K5*). O15:H1 CTX-M-14-producing strains were found in two health areas (A Coruña and Lugo) and in different years (2005 and 2007).

4.4. Clonal group O25b:H4-B2-ST131

The recent emergence of the ciprofloxacin-resistant *E. coli* O25b:H4-B2-ST131 clonal group producing CTX-M-15 and characterised by an extensive virulence

profile has been described in hospital and community settings of several countries, including Spain [4–7,27,28]. Lately, some countries have also reported the detection of this clonal group but producing CTX-M-14 [29,30]. In Europe, we first reported the detection of clone O25b:H4-ST131 producing CTX-M-14 in one strain isolated in Lugo [4]. Now we report the emergence of this CTX-M-14-producing clonal group since it was detected in three different health areas (A Coruña, Lugo and Ourense) throughout 2006–2008. Fig. 2 shows again [4,6,20] the high genetic diversity among clonal group CTX-M-producing O25b:H4-B2-ST131. This clonal group of concern has become progressively more extensive and rapidly accumulates VFs and associated antimicrobial resistances.

4.5. New clonal groups O20:H34/HNM-D-ST354 and ONT:H21,42-B1-ST101

To our knowledge, this is the first study to characterise the CTX-M-14-producing clonal groups O20:H34/HNM-D-ST354 and ONT:H21,42-B1-ST101. Previously, Oteo et al. [7] described the detection of 1 ST101-B1 strain among 41 CTX-M-14-producing strains isolated in Spain, and Lee et al. [31] detected ST101-B1 and ST354-D (two and four strains, respectively) among ciprofloxacin-resistant *E. coli* strains isolated from UTIs in South Korea. In the present study, both clonal groups showed characteristic profiles of VFs and antibiotic resistances. Both satisfied the ExPEC status and were ciprofloxacin-resistant. They were only detected in one health area (Lugo) in 2007 (O20:H34/HNM-D-ST354) and 2008 (ONT:H21,42-B1-ST101).

5. Conclusion

The emergence of multidrug-resistant virulent clonal groups of *E. coli* that may be spreading rapidly in the community poses a significant public health threat. This has been detected in this health area in northwest of Spain, since certain CTX-M-14-producing *E. coli* clones are presently emerging and some of them are already known as clonal groups of clinical importance (such as O1:HNM-D-ST59, O15:H1-D-ST393 and O25b:H4-B2-ST131). We now report important new significance of CTX-M-14-producing clones. We have also detected two new CTX-M-14-producing clonal groups (O20:H34/HNM-D-ST354 and ONT:H21,42-B1-ST101). These five clonal groups showed a high virulence potential since they harboured more than eight virulence factors, which could explain their successful dissemination.

Implementation of appropriate surveillance systems is urgently required, especially for those clonal groups that show rapid accumulation of VFs and increasing antimicrobial resistances.

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Competing interests

None declared.

Ethical approval

Not required.

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References

- [1] Matsumoto Y, Ikeda F, Kamimura T, Yokota Y, Mine Y. Novel plasmid-mediated β -lactamase from *Escherichia coli* that inactivates oxyimino-cephalosporins. *Antimicrob Agents Chemother* 1988;32:1243–6.
- [2] Pałucha A, Mikiewicz B, Hryniewicz W, Gniadkowski M. Concurrent outbreaks of extended-spectrum β -lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. *J Antimicrob Chemother* 1999;44:489–99.
- [3] Cantón R, Coque TM. The CTX-M β -lactamase pandemic. *Curr Opin Microbiol* 2006;9:466–75.
- [4] Blanco M, Alonso MP, Nicolas-Chanoine MH, Dahbi G, Mora A, Blanco JE, 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.
- [5] Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β -lactamase CTX-M-15. *Emerg Infect Dis* 2008;14:195–200.
- [6] Nicolas-Chanoine MH, Blanco J, Leflond-Guibout V, Demarty R, Alonso MP, Caniça M, et al. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008;61:273–81.
- [7] Oteo J, Navarro C, Cercenado E, Delgado-Iribarren A, Wilhelmi I, Orden B, et al. Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. *J Clin Microbiol* 2006;44:2359–66.

- [8] Bou G, Cartelle M, Tomas M, Canle D, Molina F, Moure R, et al. Identification and broad dissemination of the CTX-M-14 β -lactamase in different *Escherichia coli* strains in the northwest area of Spain. *J Clin Microbiol* 2002;40:4030–6.
- [9] Oteo J, Diestra K, Juan C, Bautista V, Novais A, Pérez-Vázquez M, 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.
- [10] Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β -lactamases conferring transferable resistance to newer β -lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988;10:867–78.
- [11] National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disc susceptibility testing*. Fourteenth informational supplement. Document M100-S14. Wayne, PA: NCCLS; 2004.
- [12] Leflon-Guibout V, Jurand C, Bonacorsi S, Espinasse F, Guelfi MC, Duportail F, et al. Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. *Antimicrob Agents Chemother* 2004;48:3736–42.
- [13] Guinée PAM, Jansen WH, Wadström T, Sellwood R. *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In: de Leew PW, Guinée PAM, editors. *Laboratory diagnosis in neonatal calf and pig diarrhoea: current topics in veterinary medicine and animal science*. London, UK: Springer; 1981.

- [14] Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, 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.
- [15] Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000;66:4555–8.
- [16] Martinez-Medina M, Mora A, Blanco M, López C, Alonso MP, Bonacorsi S, et al. Similarity and divergence among adherent-invasive *Escherichia coli* and extraintestinal pathogenic *E. coli* strains. *J Clin Microbiol* 2009;47:3968–9.
- [17] Mora A, López C, Dabhi G, Blanco M, Blanco JE, Alonso MP, et al. Extraintestinal pathogenic *Escherichia coli* O1:K1:H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. *BMC Microbiol* 2009;9:132.
- [18] Le Bouguéne C, Lalioui L, du Merle L, Jouve M, Courcoux P, Bouzari S, et al. Characterization of AfaE adhesins produced by extraintestinal and intestinal human *Escherichia coli* isolates: PCR assays for detection of Afa adhesins that do or do not recognize Dr blood group antigens. *J Clin Microbiol* 2001;39:1738–45.
- [19] Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrob Agents Chemother* 2003;47:2161–8.
- [20] Cortés P, Blanc V, Mora A, Dahbi G, Blanco JE, Blanco M, et al. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl Environ Microbiol* 2010;76:2799–805.

- [21] Valverde A, Cantón R, Garcillán-Barcia MP, Novais A, Galán JC, Alvarado A, et al. Spread of *bla*_{CTX-M-14} is driven mainly by IncK plasmids disseminated among *Escherichia coli* phylogroups A, B1, and D in Spain. *Antimicrob Agents Chemother* 2009;53:5204–12.
- [22] Oteo J, Cercenado E, Cuevas O, Bautista V, Delgado-Iribarren A, Orden B, et al. AmpC β -lactamases in *Escherichia coli*: emergence of CMY-2-producing virulent phylogroup D isolates belonging mainly to STs 57, 115, 354, 393, and 420, and phylogroup B2 isolates belonging to the international clone O25b-ST131. *Diagn Microbiol Infect Dis* 2010;67:270–6.
- [23] Corvec S, Crémet L, Leprince C, Dauvergne S, Reynaud A, Lepelletier D, et al. Epidemiology of *Escherichia coli* clinical isolates producing AmpC plasmidic β -lactamase during a 5-year period in a French teaching Hospital. *Diagn Microbiol Infect Dis* 2010;67:277–81.
- [24] Phillips I, Eykyn S, King A, Gransden WR, Rowe B, Frost JA, et al. Epidemic multiresistant *Escherichia coli* infection in West Lambeth Health District. *Lancet* 1988;1:1038–41.
- [25] Cagnacci S, Gualco L, Debbia E, Schito GC, Marchese A. European emergence of ciprofloxacin-resistant *Escherichia coli* clonal groups O25:H4-ST131 and O15:K52:H1 causing community-acquired uncomplicated cystitis. *J Clin Microbiol* 2008;46:2605–12.
- [26] Olesen B, Scheutz F, Menard M, Skov MN, Kolmos HJ, Kuskowski MA, et al. Three-decade epidemiological analysis of *Escherichia coli* O15:K52:H1. *J Clin Microbiol* 2009;47:1857–62.

- [27] Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M β -lactamases: the worldwide emergence of clone ST131 O25:H4. *Int J Antimicrob Agents* 2010;35:316–21.
- [28] Díaz MA, Hernández-Bello JR, Rodríguez-Baño J, Martínez-Martínez L, Calvo J, Blanco J, et al. Diversity of *Escherichia coli* strains producing extended-spectrum β -lactamases in Spain: second nationwide study. *J Clin Microbiol* 2010;48:2840–5.
- [29] Pitout JD, Gregson DB, Church DL, Elsayed S, Laupland KB. Community-wide outbreaks of clonally related CTX-M-14 β -lactamase-producing *Escherichia coli* strains in the Calgary health region. *J Clin Microbiol* 2005;43:2844–9.
- [30] Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y. Change in the prevalence of extended-spectrum- β -lactamase-producing *Escherichia coli* in Japan by clonal spread. *J Antimicrob Chemother* 2009;63:72–9.
- [31] Lee MY, Choi HJ, Choi JY, Song M, Song Y, Kim SW, et al. Dissemination of ST131 and ST393 community-onset, ciprofloxacin-resistant *Escherichia coli* clones causing urinary tract infections in Korea. *J Infect* 2010;60:146–53.

Fig. 1. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested DNA from 32 CTX-M-14-producing *Escherichia coli* strains included in the present study. The dendrogram was produced by the unweighted pair group method using arithmetic averages (UPGMA) algorithm based on Dice similarity coefficient with a 1.0% band position tolerance. Strain designation, serotype, phylogenetic group, sequence type (ST), date, hospital of origin, virulence genes and associated resistances are shown on the right-hand side. Strain FV 10827 (O25b:H4) produced both CTX-M-14 and CTX-M-15. CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; TOB, tobramycin.

Fig. 2. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested DNA from 14 *Escherichia coli* strains belonging to clonal group O25b:H4-B2-ST131. The dendrogram was produced by the unweighted pair group method using arithmetic averages (UPGMA) algorithm based on Dice similarity coefficient with a 1.0% band position tolerance in order to compare the five CTX-M-14 O25b:H4 strains included in the present study with other strains of the same serotype producing different CTX-M-types. Strain designation, CTX-M-type, virulence genes and associated resistances are shown on the right-hand side. CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim/sulfamethoxazole.

Table 1

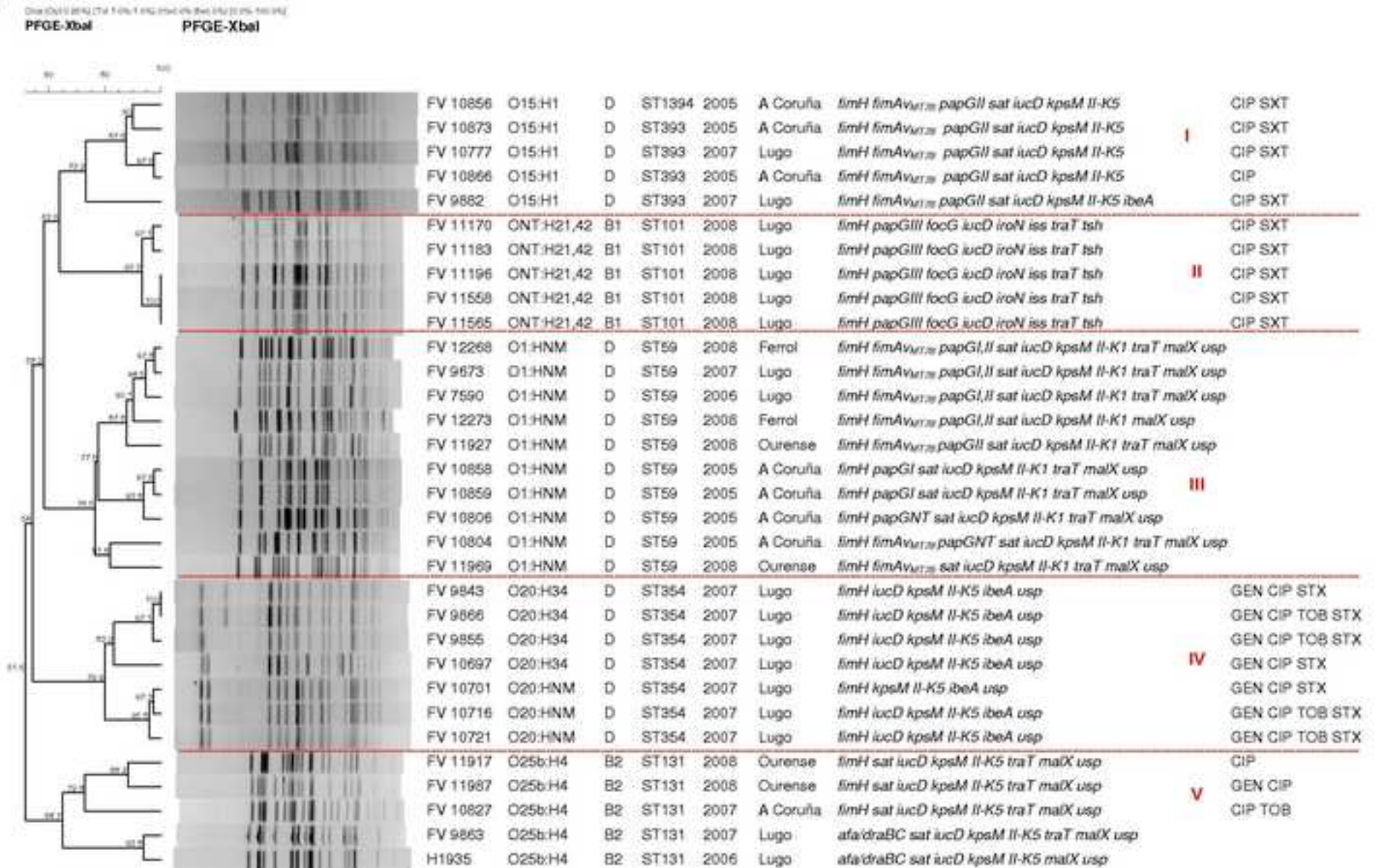
Virulence gene characterisation of the five clonal groups

Category/gene(s)	Comment	O1:HNM-D-ST59 (<i>n</i> = 10)	O15:H1-D-ST393/ST1394 (<i>n</i> = 5)	O20:H34/HNM-D-ST354 (<i>n</i> = 7)	O25b:H4-B2-ST131 (<i>n</i> = 5)	ONT:H21,42-B1-ST101 (<i>n</i> = 5)
Adhesins						
<i>fimH</i>	D-mannose-specific adhesin, type 1 fimbriae	10	5	7	3	5
<i>fimA_{MT78}</i>	FimA variant MT78 of type 1 fimbriae	7	5	0	0	0
<i>pap</i>	Pilus associated with pyelonephritis (P fimbriae)					
<i>papEF</i>	Pilus assembly; central region of <i>pap</i> operon	9	5	0	0	5
<i>papG</i>	Gal(α 1–4) Gal-specific pilus tip adhesin molecule	9	5	0	0	5
<i>papG I</i>	Rare	6	0	0	0	0

<i>papG II</i>	Pyelonephritis-associated	5	5	0	0	0
<i>papG III</i>	Cystitis-associated	0	0	0	0	5
<i>sfa/focDE</i>	Central region of <i>sfa</i> (S fimbriae) and <i>foc</i> (F1C fimbriae) operons	0	0	0	0	5
<i>sfaS</i>	Pilus tip adhesin, S fimbriae (sialic acid-specific)	0	0	0	0	0
<i>focG</i>	Pilus tip molecule, F1C fimbriae (sialic acid-specific)	0	0	0	0	5
<i>afa/draBC</i>	Dr antigen-specific adhesin operons (AFA, Dr, F1845)	0	0	0	2	0
<i>bmaE</i>	Blood group M-specific adhesin	0	0	0	0	0
<i>gafD</i>	<i>N</i> -acetyl-D-glucosamine-specific (G, F17c) fimbriae adhesin	0	0	0	0	0

Toxins						
<i>cnf1</i>	Cytotoxic necrotizing factor 1	0	0	0	0	0
<i>cdtB</i>	Cytolethal distending toxin	0	0	0	0	0
<i>sat</i>	Secreted autotransporter toxin	10	5	0	5	0
<i>hlyA</i>	α -haemolysin	0	0	0	0	0
Siderophores						
<i>iucD</i>	Ferric aerobactin receptor (iron uptake: transport)	10	5	6	5	5
<i>iroN</i>	Novel catecholate siderophore receptor	0	0	0	0	5
Protection						
<i>kpsM II</i>	Group II capsule	10	5	7	5	0
<i>kpsM II-K2</i>	K2 group II capsule	0	0	0	0	
<i>kpsM II-K5</i>	K5 group II capsule	0	5	7	5	
<i>neuC-K1</i>	K1 antigen	10	0	0	0	0
<i>kpsM III</i>	Group III capsule	0	0	0	0	0

<i>cvaC</i>	ColV; on plasmids with <i>traT</i> , <i>iss</i> and antibiotic resistance	0	0	0	0	0
<i>iss</i>	Increased serum survival (outer membrane protein)	0	0	0	0	5
<i>traT</i>	Surface exclusion, serum survival (outer membrane protein)	9	0	0	4	5
Miscellaneous						
<i>ibeA</i>	Invasion of brain endothelium IbeA	0	1	7	0	0
<i>malX</i>	Pathogenicity-associated island (PAI) marker	10	0	0	5	0
<i>usp</i>	Uropathogenic-specific protein (bacteriocin)	10	0	7	5	0
<i>tsh</i>	Tsh (temperature-sensitive haemagglutinin) serine protease	0	0	0	0	5



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