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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 Complexo Hospitalario Xeral-Calde, Lugo (325 strains), Complexo Hospitalario Universitario A Coruña (150 strains), Complexo Hospitalario de Ourense (100 strains) and Complexo Hospitalario Arquitecto Marcide-Prof. Novoa Santos, Ferrol (79 strains). The strains included in the present study (one strain

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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 (*adk*, *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 Bouguénec 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 $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ 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 Complexo 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