Extended-spectrum β-lactamase-producing in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2

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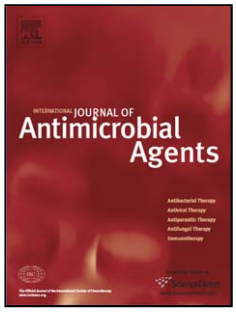

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Extended-spectrum β-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2

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Abstract

In this study, we investigated the population structure of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in Spain and determined possible associations between specific multilocus sequence typing (MLST) types and ESBL types. Ninety-two ESBL-producing *E. coli* isolates from 11 Spanish hospitals were studied. The predominant ESBLs in this collection were CTX-M-14 (45.7%), SHV-12 (21.7%) and CTX-M-9 (20.6%). Phylogenetic groups and MLST types were studied. Thirty-seven isolates (40.2%) belonged to phylogroup A, 26 (28.3%) to group B1, 13 (14.1%) to group B2 and 16 (17.4%) to group D. Fifty-six sequence types (STs) were identified, but ST131 (eight isolates) and ST167 (five isolates) were the most prevalent. The most common ST complexes were ST10 (13 isolates; 14.3%) and ST23 (10 isolates; 11%). *Escherichia coli* ST131 carried six different ESBLs (CTX-M-1, CTX-M-9, CTX-M-10, CTX-M-14, CTX-M-15 and SHV-12), *E. coli* ST10 complex carried five ESBLs and *E. coli* ST23 complex carried four ESBLs. A great diversity of MLST types was observed among Spanish ESBL-producing *E. coli* isolates.

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1. Introduction

Most European countries have experienced a recent rapid dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates, in particular CTX-M-type [1,2]. In Spanish invasive *E. coli* isolates, ESBL production increased from 1.6% in 2001 to 7% in 2007 (European Antimicrobial Resistance Surveillance System; http://www.rivm.nl/earss/). Although the spread of ESBLs is frequently due to the dissemination of mobile genetic elements [2–4], clonal dissemination of CTX-M-15-producing *E. coli* belonging to phylogenetic group B2 and ST131 has been addressed in different countries [5–8]. In addition, clonal spread of the CTX-M-9-group-producing ST38 strain has been described in a recent study in Japan [9]. The various methods by which ESBLs spread greatly influence the prevalence of different enzymes in specific areas.

Multilocus sequence typing (MLST) is expensive and time consuming, and almost all studies that characterise the population structure of ESBL-producing *E. coli* have selected the study population by some other criteria, such as serotyping [9] or production of specific ESBLs, e.g. CTX-M-15 [5,8]. As this approach may produce biased results, we sought to investigate the population genetics of *E. coli* carrying a variety of ESBLs without any previous selection.

Our study aimed to determine (i) the population structure of ESBL-producing *E. coli* in Spain and (ii*)* the possible association between specific MLST types and ESBL types. To attain these objectives, the population structure of a large, well

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characterised collection of 92 ESBL-producing *E. coli* isolates collected during a Spanish survey [10] was characterised.

2. Material and methods

A collection of 92 ESBL-producing *E. coli* isolates was recovered from clinical samples (68.5% from urine) from 11 Spanish hospitals in five geographic regions (Andalucia, Cataluña, Madrid, Baleares and Valencia). All hospitals were members of the Spanish Network for Research in Infectious Diseases (REIPI) [10]. Participant hospitals were asked to collect up to 15 consecutive, non-duplicate (one isolate per patient) ESBL-producing *E. coli* isolates by the first quarter of 2004. Eight different ESBL types were identified, with the predominant types being CTX-M-14 (45.7%), SHV-12 (21.7%) and CTX-M-9 (20.6%), as described previously [10]. In addition, the plasmids encoding bla_{ESBL} and the corresponding surrounding genes in selected isolates were further characterised [11].

In the present study, MLST types and phylogenetic groups of the ESBL-producing *E. coli* were analysed. Phylogenetic groups were determined by a multiplex polymerase chain reaction (PCR) assay as described by Clermont et al. [12]. Analysis of two genes (*chuA* and *yjaA*) and an anonymous DNA fragment (TSPE4.C2) allowed the identification of the four major phylogenetic groups of *E. coli.*

MLST determinations were carried out according to the Max-Planck Institute scheme for *E. coli* developed by M. Achtman et al. (http://web.mpiib-

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berlin.mpg.de). The phylogenetic relationships among the different sequence types (STs) found in this study were established according to the eBURST program version 2 (http://linux.mlst.net/burst.htm). STs of all *E. coli* isolates from the MLST database as well as STs of ESBL-producing *E. coli* from this study were considered together and introduced into the eBURST for analysis and elaboration of a population snapshot (Fig. 1).

Statistical analyses were performed using GraphPad Prism version 3.02 software (GraphPad Software Inc., San Diego, CA).

3. Results

3.1. Phylogenetic groups

Of the 92 ESBL-producing *E. coli* isolates, 37 (40.2%) belonged to phylogenetic group A, 26 (28.3%) to group B1, 13 (14.1%) to group B2 and 16 (17.4%) to group D. Seven different ESBL types were found among *E. coli* phylogroup A isolates, four among phylogroup B1 isolates, six among phylogroup B2 isolates and three among phylogroup D isolates.

Escherichia coli producing the three most prevalent ESBL types (CTX-M-14, CTX-M-9 and SHV-12) were distributed among all four phylogroups, with CTX-M-14-producing isolates being the most prevalent in all of them. Of the three CTX-M-15-producing isolates, two were phylogroup B2 and one was phylogroup A.

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3.2. MLST analysis

Among the 91 isolates tested (1 isolate could not be sequenced), 56 different STs were identified. The most prevalent STs were ST131 (eight isolates), ST167 (five isolates), ST23 (four isolates), ST602 (four isolates) and ST359 (four isolates) (Fig. 1; Table 1). The ST131 isolates were recovered from different geographic locations: Barcelona (four isolates from three different hospitals); Madrid (three isolates from one hospital); and Seville (one isolate).

By eBURST, 33 STs were distributed into 15 different ST complexes (53 isolates), whilst 23 STs were considered singletons (38 isolates) (Table 1). The two main ST complexes were ST10 (13 isolates; 14.3%) and ST23 (10 isolates; 11%). Five of the eight most frequent STs, including ST131, were singletons (Table 1).

Analysis with eBURST provided an overview of STs of the Spanish ESBL-producing *E. coli* isolates from this study compared with all *E. coli* isolates from the MLST database. The major ST complexes found are represented in Fig. 1. ST10 complex was the most commonly detected in this study (14.3%) and is also the most frequent ST in the MLST database (9.4%).

3.3. Sequence type distribution in relation to extended-spectrum β-lactamase types

The Spanish ESBL-producing *E. coli* were distributed among a large number of MLST types (Table 1). The CTX-M-14-producers were distributed into 12 different ST complexes (28 isolates) and 10 singletons (13 isolates) (Table 1).

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The SHV-12-producers alone were distributed among six ST complexes (11 isolates) and 7 singletons (7 isolates) (Table 1). The CTX-M-9-producers alone were distributed among four ST complexes (8 isolates) and 7 singletons (9 isolates) (Table 1).

Escherichia coli ST131 isolates carried six different ESBLs: CTX-M-9; CTX-M-10; CTX-M-1; CTX-M-14; CTX-M-15; and SHV-12. In addition, *E. coli* isolates belonging to the two major complexes, ST10 and ST23, had five and four ESBL types, respectively.

3.4. Phylogenetic groups in relation to sequence types

A strong association between STs and phylogenetic groups was found, as all 23 isolates of the ST10 and ST23 complexes were phylogroup A. Also, of eight ST131 isolates, seven belonged to the virulent extraintestinal phylogroup B2, and the three ST117 isolates belonged to phylogroup D (Table 1).

4. Discussion

We characterised the population structure of 92 *E. coli* isolates carrying eight types of ESBLs, seven of the CTX-M family. Several findings are of epidemiological and microbiological interest: (i) a great diversity of MLST types was observed; (ii) the *E. coli* isolates belonging to the most common STs or ST complexes could carry almost all of the ESBL types identified in this study; and (iii) ST10 complex (the most frequent in this study and also in the MLST

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database) carried five different ESBLs (CTX-M-14, SHV-12, CTX-M-9, CTX-M-15 and CTX-M-32).

The spread of ST131/B2 CTX-M-15-producing *E. coli* isolates, previously described in Madrid, Spain [5], and in other countries [6–8], was not observed in this study. In fact, of the three CTX-M-15-producing isolates, only one was ST131/B2 whilst the other two belonged to STs 167 and 653. Plasmids that carry CTX-M-15 are well disseminated among circulating virulent ST131/B2 *E. coli* strains in some geographic areas [6–8,13]; however, we found that ST131 *E. coli* not only carried CTX-M-15 but also CTX-M-9, CTX-M-10, CTX-M-14, SHV-12 and CTX-M-1. These results agree with the very recent description of ST131 *E. coli* harbouring $bla_{\text{CTX-M-2-group}}$ and $bla_{\text{CTX-M-14}}$ in Japan [9]. Unlike the epidemic ST38 *E. coli* described in Japan that is associated with CTX-M-9-group ESBLs [9], we detected only one ST38 isolate producing CTX-M-14.

In a recent French study, ST131 and ST10 complex isolates were found to be the most prevalent among faecal samples from healthy carriers of nalidixic acid-resistant (but ESBL-negative) *E. coli* [14]. In England, 59% of uropathogenic *E. coli* were ST131 (98.1% of them resistant to cefpodoxime); the next most prevalent strains, ST73 and ST95 (6.8% each) [15], were not detected in our study.

None of the ESBL-producing *E. coli* analysed in this study caused outbreaks in any of the participating Spanish hospitals. However, we have previously reported the epidemic dissemination of a clonal CTX-M-15-producing *E. coli* in

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community healthcare centres, long-term care facilities and hospitals in the Madrid area [5]. Two epidemiological scenarios are possible: the clonal spread of a few specific strains; and the dissemination of bla_{ESBL} genes among a diverse population of *E. coli*. The two models can occur simultaneously or separately, thereby contributing to the rapid dissemination of ESBLs.

In this study, we characterised the population structure of 92 *E. coli* isolates carrying eight ESBL types, with seven from the CTX-M family. Although a diversity of MLST types was observed, *E. coli* belonging to the most common STs or ST complexes (ST131, ST10 complex and ST23 complex) can carry almost all of the ESBL types. *Escherichia coli* ST131 carried not only CTX-M-15 but also CTX-M-9, CTX-M-10, CTX-M-14, SHV-12 and CTX-M-1.

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Fig. 1. Population snapshot of sequence types (STs) of all *Escherichia coli* isolates from the multilocus sequence typing (MLST) database and STs of extended-spectrum β-lactamse (ESBL)-producing *E. coli* from this study considered altogether. The most important ST complexes found in this study (ST10 complex, ST23 complex, ST31 complex, ST131, ST446 complex and ST538 complex) are emphasised.

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Table 1

Distribution of sequence type (ST) and phylogenetic groups among 91 extended-spectrum β-lactamse (ESBL)-producing *Escherichia coli* isolates in relation to ESBL type

ESBL (*n*)	ST complex (*n*)	ST [a] (phylogenetic group)
CTX-M-14 (41)	ST23 (6)	23[2] (A); 88[1] (A); 410[1] (A); 612[1] (A); 650[1] (A)
	ST10 (4)	617[2] (A); 48[1] (A); 167[1] (A)
	ST31 (3)	130[1] (D); 393[1] (D); 613[1] (D)
	ST538 (3)	538[2] (D); 620[1] (B2)
	ST46 (2)	615[2] (A)
	ST101 (2)	101[1] (B1); 619[1] (B1)
	ST155 (2)	616[1] (B1); 621[1] (B1)
	ST156 (2)	348[1] (B1); 611[1] (B1)
	ST38 (1)	38[1] (D)
	ST69 (1)	69[1] (D)
	ST350 (1)	57[1] (A)
	ST354 (1)	623[1] (B2)
	Singletons (13)	359[3] (B1); 224[2] (B1); 131[1] (B2); 196[1] (B1); 610[1] (B1); 614[1] (A); 622[1] (B2); 624[1] (D); 625[1] (B2); 649[1] (B1)
SHV-12 (18)	ST10 (4)	167[2] (A); 10[1] (A); 227[1] (A)
	ST23 (2)	23[2] (A)
	ST168 (2)	93[2] (A)
	ST101 (1)	604[1] (B1)
	ST350 (1)	57[1] (D)
	ST354 (1)	354[1] (D)
	Singletons (7)	117[1] (D); 127[1] (D); 131[1] (B2); 224[1] (B1); 297[1] (B1); 605[1] (A); 608[1] (A)
CTX-M-9 (17)	ST446 (4)	602[4] (B1)

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	ST10 (2)	193[2] (A)
	ST59 (1)	59[1] (D)
	ST156 (1)	156[1] (B1)
	Singletons (9)	117[2] (D); 131[2] (B2, D); 359[1] (B1); 401[1] (A); 600[1] (B1); 606[1] (B1); 607[1] (A)
Other ESBL (15) [b]	ST10 (3)	167[2] (A); 10[1] (A)
	ST23 (2)	88[1] (A); 410[1] (A)
	ST46 (1)	609[1] (A)
	Singletons (9)	131[4] (B2); 401[2] (A); 540[1] (A); 603[1] (B1); 653[1] (B2)

[a] Number of isolates in superscript.

[b] Two CTX-M-1, three CTX-M-3, two CTX-M-10, three CTX-M-15, three CTX-M-32 and two CTX-M-9 + SHV-12.

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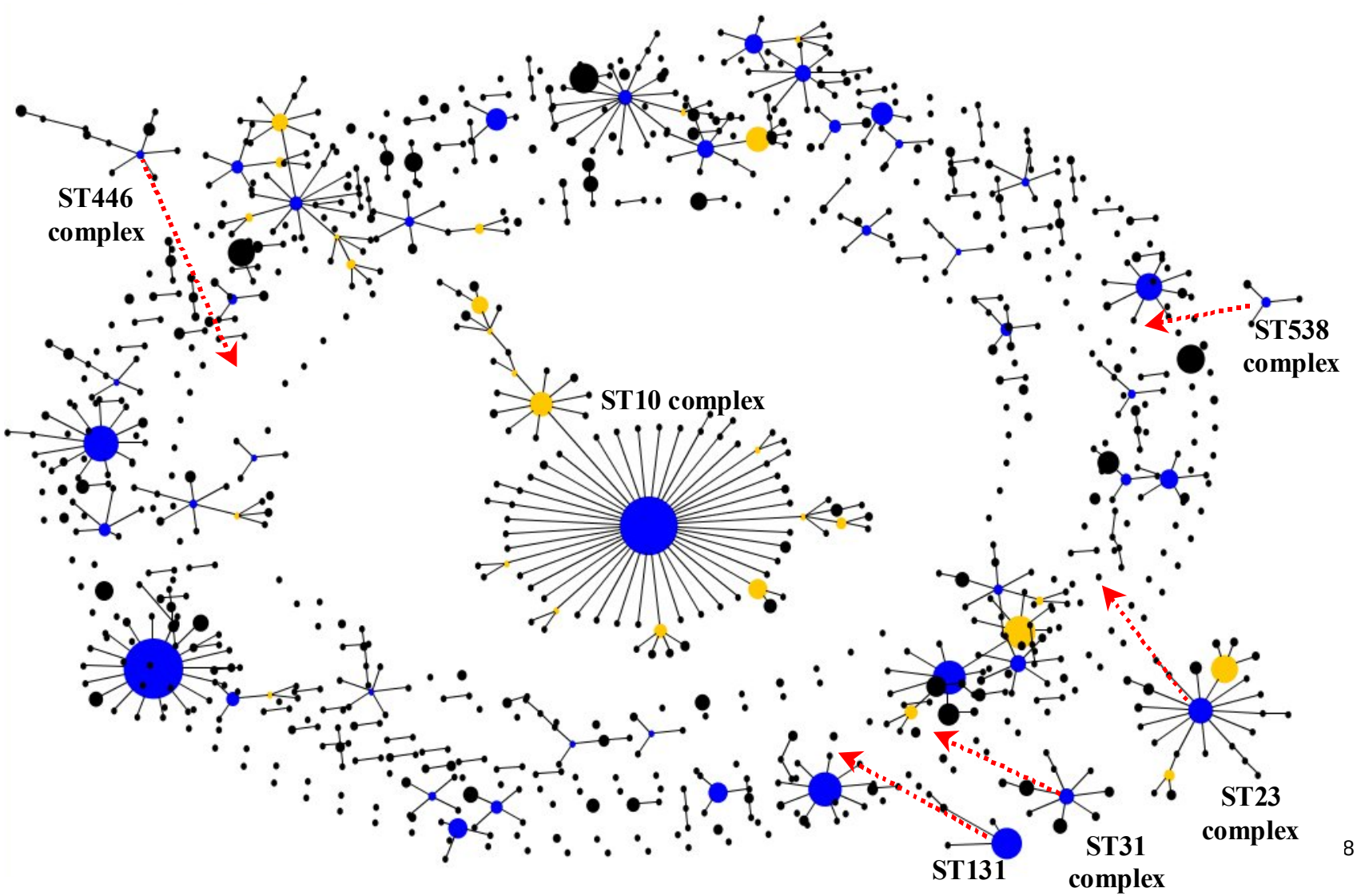
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