

National survey of *Escherichia coli* causing extraintestinal infections reveals the spread of drug-resistant clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69 with high virulence gene content in Spain

Jorge Blanco^{1*}†, Azucena Mora¹†, Rosalía Mamani¹†, Cecilia López¹, Miguel Blanco¹, Ghizlane Dahbi¹, Alexandra Herrera¹, Jesús E. Blanco¹, María Pilar Alonso², Fernando García-Garrote², Fernando Chaves³, María Ángeles Orellana³, Luis Martínez-Martínez^{4,5}, Jorge Calvo^{4,5}, Guillem Prats^{6,7}, María Nieves Larrosa^{6,7}, Juan José González-López^{6,7}, Lorena López-Cerero⁸, Jesús Rodríguez-Baño^{9,10} and Álvaro Pascual^{8,11}

¹Laboratorio de Referencia de *E. coli* (LREC), Departamento de Microbiología e Parasitología, Facultad de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain; ²Unidad de Microbiología Clínica, Hospital Lucus Augusti, Lugo, Spain; ³Servicio de Microbiología, Hospital 12 de Octubre, Madrid, Spain; ⁴Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla-IFIMAV, Santander, Spain; ⁵Departamento de Biología Molecular, Universidad de Cantabria, Santander, Spain; ⁶Servei de Microbiologia, Hospital Vall d'Hebron, Barcelona, Spain; ⁷Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Spain; ⁸Servicio de Microbiología, Hospital Universitario Virgen Macarena, Sevilla, Spain; ⁹Unidad de Enfermedades Infecciosas, Hospital Universitario Virgen Macarena, Sevilla, Spain; ¹⁰Departamento de Medicina Interna, Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain; ¹¹Departamento de Microbiología, Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain

*Corresponding author. Tel/Fax: +34-982-822108; E-mail: jorge.blanco@usc.es

†These authors made equal contributions to this study.

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Objectives: To evaluate the current prevalence of the three clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69 (where ST stands for sequence type) among *Escherichia coli* isolates causing extraintestinal infections in Spain and to characterize their virulence background, 500 consecutive non-duplicate *E. coli* isolates causing extraintestinal infections were analysed.

Methods: The 500 isolates were collected during February 2009 from five hospitals in different Spanish regions. Phylogenetic groups, STs, serotypes, virulence genes, PFGE profiles, antimicrobial resistance and extended-spectrum β -lactamase (ESBL) enzymes were determined.

Results: The three clonal groups accounted for 19% of the 500 isolates. Furthermore, they accounted for 37% of the isolates exhibiting trimethoprim/sulfamethoxazole plus ciprofloxacin resistance, 34% of aminoglycoside-resistant isolates and 30% of multidrug-resistant isolates. Clonal group ST131 was the most prevalent, and accounted for 12% of isolates overall and for 23% of multidrug-resistant isolates. The ST131 isolates exhibited a significantly higher virulence score (mean of virulence genes 8.1) compared with the ST393 (6.0) and ST69 (5.4) isolates. The prevalence of ESBL-producing isolates was 7%. Six (10%) of the 59 ST131 isolates were positive for CTX-M-15 and one (6%) of the 16 ST393 isolates was positive for CTX-M-14, whereas none of the 22 ST69 isolates produced ESBL enzymes.

Conclusions: The three clonal groups investigated accounted for 30% of the multidrug-resistant isolates, which gives evidence of an important clonal component in the emergence of resistances among extraintestinal pathogenic *E. coli*. Notably, a single high virulence clonal group (O25b:H4-B2-ST131) causes approximately 1 in every 10 extraintestinal infections in Spain, representing an important public health threat. A new variant of the ST131 clonal group, which is non-ESBL-producing but trimethoprim/sulfamethoxazole resistant and with high virulence content, is reported.

Keywords: *E. coli*, ExPEC, antimicrobial resistance

Introduction

The intercontinental *Escherichia coli* clonal group producing CTX-M-15 with a high virulence potential, O25b:H4-B2-ST131 (where ST stands for sequence type), has been reported recently all over the world, representing a major public health problem.^{1,2} This clonal group belongs to the B2 phylogenetic group, to the serotype O25b:H4 and to the multilocus ST131. The O25b:H4-B2-ST131 clonal group is characterized by co-resistance to several classes of antibiotics and is able to acquire different mechanisms of resistance. Although commonly associated with the dissemination of CTX-M-15 extended-spectrum cephalosporin resistance, *E. coli* O25b:H4-B2-ST131 also occurs as a fluoroquinolone-resistant but cephalosporin-susceptible pathogen.^{3–12}

Since the outbreak of 1986 in London, numerous studies have reported evidence of the continued clinical importance and global distribution of another fluoroquinolone-resistant clonal group that belongs to the D phylogenetic group, to the serotype O15:H1 and to the multilocus ST393.^{4,6,13,14} Olesen *et al.*¹⁵ recently published an epidemiological analysis with the conclusion that the O15:H1-D-ST393 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. Similarly, Mora *et al.*¹⁶ recently reported the emergence of CTX-M-14-producing isolates of this clonal group in Spain.

Clonal group A (CGA) belongs to the D phylogenetic group and to the multilocus ST69, and the isolates exhibit distinctive O groups (O11, O17, O73 and O77). CGA-D-ST69 accounted for up to 50% of trimethoprim/sulfamethoxazole-resistant urinary tract infections (UTIs) due to *E. coli* in the USA in the 1990s and also has a worldwide distribution.^{6,17–20}

Despite the clinical importance and worldwide distribution of these three drug-resistant clonal groups, as far as we know, they have been screened in only one study carried out in Canada from 2002 to 2004.⁶ For this reason, the present study was carried out to evaluate the current prevalence of these three clonal groups among *E. coli* isolates causing extraintestinal infections in Spain and to understand their contribution to the emergence of antimicrobial resistance. This type of study has a special interest due to the dramatic increase in ciprofloxacin and trimethoprim/sulfamethoxazole resistances observed in Spain during recent years.^{21,22}

Materials and methods

Bacterial isolates

Five hospitals in different Spanish regions (Lugo, LU; Barcelona, BA; Santander, SA; Madrid, MA; and Seville, SE) participated in the present study. In the study period (February 2009), 100 consecutive non-duplicate clinically relevant *E. coli* isolates were obtained in each hospital from ambulatory (71%) and hospital-admitted (29%) patients. Clinical sources included urine (421 isolates), blood (25 isolates), surgical wounds (14 isolates), respiratory tract samples (5 isolates), bile (5 isolates) and other sources (30 isolates).

Antimicrobial susceptibility and extended-spectrum β -lactamase (ESBL) typing

Susceptibility to antibiotics was analysed by broth microdilution and/or disc diffusion. Minimal inhibitory concentrations were determined using

a MicroScan WalkAway automated system (Siemens, Madrid, Spain) according to the manufacturer's instructions. Intermediate susceptibility was not considered as resistant. Resistance was interpreted based on the recommended breakpoints of the CLSI.²³ Multidrug-resistant isolates were those resistant to at least one representative of three or more antimicrobial classes, including fluoroquinolones (ciprofloxacin), trimethoprim/sulfamethoxazole, aminoglycosides (gentamicin and tobramycin), β -lactam/ β -lactamase inhibitors (amoxicillin/clavulanic acid and piperacillin/tazobactam) and extended-spectrum cephalosporins (cefotaxime, ceftazidime and cefepime).

Suggestive evidence of ESBL production was defined as synergy between amoxicillin/clavulanate and at least one of cefotaxime, ceftazidime, aztreonam or cefepime. To determine the genotype of the ESBLs, PCR was performed using the TEM, SHV, CTX-M-1 and CTX-M-9 group-specific primers, as reported previously.^{7,24} Sequencing of both strands of amplicons was performed using specific primers.

Detection of clonal groups

For detection of the three drug-resistant clonal groups, all 500 isolates were screened by PCR for the O25b *rfb* variant (O25b:H4-B2-ST131 associated),²⁵ for the O15 *rfb* allele and a single nucleotide polymorphism (SNP) in *fumC* specific for CC31 (where CC stands for clonal complex) (O15:H1-D-ST393 associated),^{6,26} and for an SNP in *fumC* and *gyrB* specific for CC69 (CGA-D-ST69 associated).^{6,27}

Phylogenetic grouping and multilocus sequence typing (MLST)

To confirm the presumptive clonal group assignments, all isolates of the three clonal groups detected by the PCR screening were analysed for their phylogenetic group (A, B1, B2 and D) by the multiplex PCR-based method of Clermont *et al.*²⁸ and MLST. MLST was achieved as previously described by gene amplification and sequencing of the seven housekeeping genes (*adh*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) according to the protocol and primers specified at the *E. coli* MLST web site (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). The allelic profile of the seven gene sequences and the STs were obtained via the electronic database at the *E. coli* MLST web site.²⁹

O and H typing

Determination of O and H antigens was carried out using the method previously described by Guinée *et al.*³⁰ with all available O (O1–O181) and H (H1–H56) antisera. Isolates that did not react with O and H antisera were classified as non-typeable (ONT and HNT, respectively), and those non-motile were denoted as HNM. Additionally, the specific O25a and O25b molecular subtypes were determined by PCR.²⁵

Virulence factors

The presence of 30 virulence genes was analysed as documented previously,^{31–33} using primers specific for genes and operons that encode extraintestinal virulence factors characteristic of extraintestinal pathogenic *E. coli* (ExPEC), i.e. *fimH*, *fimAV_{MT78}*, *papEF* (positive results were tested for *papG I*, *papG II*, *papG III* and *papG IV* alleles), *sfa/focDE*, *afa/draBC* (positive results were tested for *afa* operon FM955459), *bmaE*, *gafD*, *cnf1*, *cdtB*, *sat*, *hlyA*, *iucD*, *iroN*, *kpsM II* (establishing *neuC*-K1, -K2 and -K5 variants), *kpsM III*, *cvaC*, *iss*, *traT*, *ibeA*, *malX*, *usp* and *tsh*.

PFGE

XbaI-PFGE analysis was performed as previously described.³¹ Profiles were analysed with the BioNumerics fingerprinting software (Applied

Maths, St-Martens-Latem, Belgium). Dendrograms were generated by the unweighted pair-group method using arithmetic averages, based on the Dice similarity coefficient with a 1.0% band position tolerance.

Statistical analysis

Comparisons of proportions and scores (continuous variables) were tested using Fisher's exact test and the Mann-Whitney *U*-test, respectively. For each comparison, a *P* value of <0.05 was considered to denote significant differences.

Results and discussion

Prevalence of clonal groups

According to molecular typing, the clonal group O25b:H4-B2-ST131 accounted for 59 (12%) of the 500 study isolates, the clonal group O15:H1-D-ST393 accounted for 16 (3%) and CGA-D-ST69 accounted for 22 (4%). Thus, the three clonal groups collectively accounted for 19% of the total isolates analysed. Hospital Vall d'Hebron in Barcelona city exhibited the highest prevalence (30%) of the three clonal groups. ST131 and ST69 occurred in all five hospitals, and ST393 in four. ST131 was significantly more prevalent than either of the other two groups in the five hospitals (*P*<0.001 for each comparison and collectively) (Table 1). The three clonal groups were detected with a similar prevalence among isolates obtained from

ambulatory and hospital-admitted patients: ST131 (12% versus 12%, respectively); ST393 (3% versus 4%, respectively); and ST69 (4% versus 4%, respectively).

In Canada during 2002–04, Johnson *et al.*⁶ found that the three clonal groups contributed substantially to the study population (37%); however, in the Canadian study four subsets of ~50 isolates (all from UTIs) were selected according to combined trimethoprim/sulfamethoxazole and fluoroquinolone phenotypes, and so it is difficult to make a detailed comparison with our results. Nevertheless, *E. coli* ST131, like in Spain, was the most prevalent clonal group in Canada (23%). In another study including mostly invasive isolates, Johnson *et al.*⁸ estimated that ST131 caused 17% of *E. coli* infections in patients hospitalized across the USA in 2007.

Distribution of the three clonal groups by resistance subgroup

In the present study, the three clonal groups accounted for 37% of the isolates exhibiting trimethoprim/sulfamethoxazole plus ciprofloxacin resistance, 34% of the aminoglycoside-resistant isolates and 30% of the multidrug-resistant isolates (Table 2). CGA-D-ST69 was concentrated within the trimethoprim/sulfamethoxazole-resistant and ciprofloxacin-susceptible group (10 of 81 isolates, 12%), whereas the O25b:H4-B2-ST131 (23%)

Table 1. Prevalence of the three clonal groups among 500 *E. coli* isolates from five hospitals

Hospital	City	Total isolates	Prevalence of clonal groups, number of isolates		
			O25b:H4-B2-ST131	O15:H1-D-ST393	CGA-D-ST69
Hospital Lucus Augusti ^a	Lugo	100	8	4	5
Hospital Vall d'Hebron	Barcelona	100	16	5	9
Marqués de Valdecilla	Santander	100	9	2	2
12 de Octubre	Madrid	100	15	0	3
Virgen Macarena	Seville	100	11	5	3
Total		500	59 (12%)	16 (3%)	22 (4%)

^aPreviously named Complejo Hospitalario Xeral-Calde.⁷

Table 2. Distribution of the three clonal groups by resistance subgroup

Resistance phenotypes ^a	Total isolates	Prevalence of clonal groups, number of isolates (%)			
		O25b:H4-B2-ST131	O15:H1-D-ST393	CGA-D-ST69	Any
Trimethoprim/sulfamethoxazole	174	31 (18%)	10 (6%)	14 (8%)	55 (32%)
Fluoroquinolones	155	41 (26%)	14 (9)	4 (3%)	59 (38%)
Trimethoprim/sulfamethoxazole plus fluoroquinolones	93	21 (23%)	9 (10%)	4 (4%)	34 (37%)
Aminoglycosides	61	17 (28%)	0	4 (7%)	21 (34%)
Amoxicillin/clavulanic acid	39	6 (15%)	0	0	6 (15%)
Extended-spectrum cephalosporins	38	6 (16%)	1 (3%)	0	7 (19%)
Any of the above	256	51 (20%)	14 (5%)	14 (5%)	79 (31%)
Multidrug resistance (three or more of the above drug classes)	61	14 (23%)	1 (2%)	3 (5%)	18 (30%)

^aFluoroquinolones, resistant to ciprofloxacin; aminoglycosides, resistant to gentamicin, tobramycin and/or amikacin; extended-spectrum cephalosporins, resistant to cefepime, ceftazidime and/or cefotaxime.

Table 3. Virulence genes of the three clonal groups

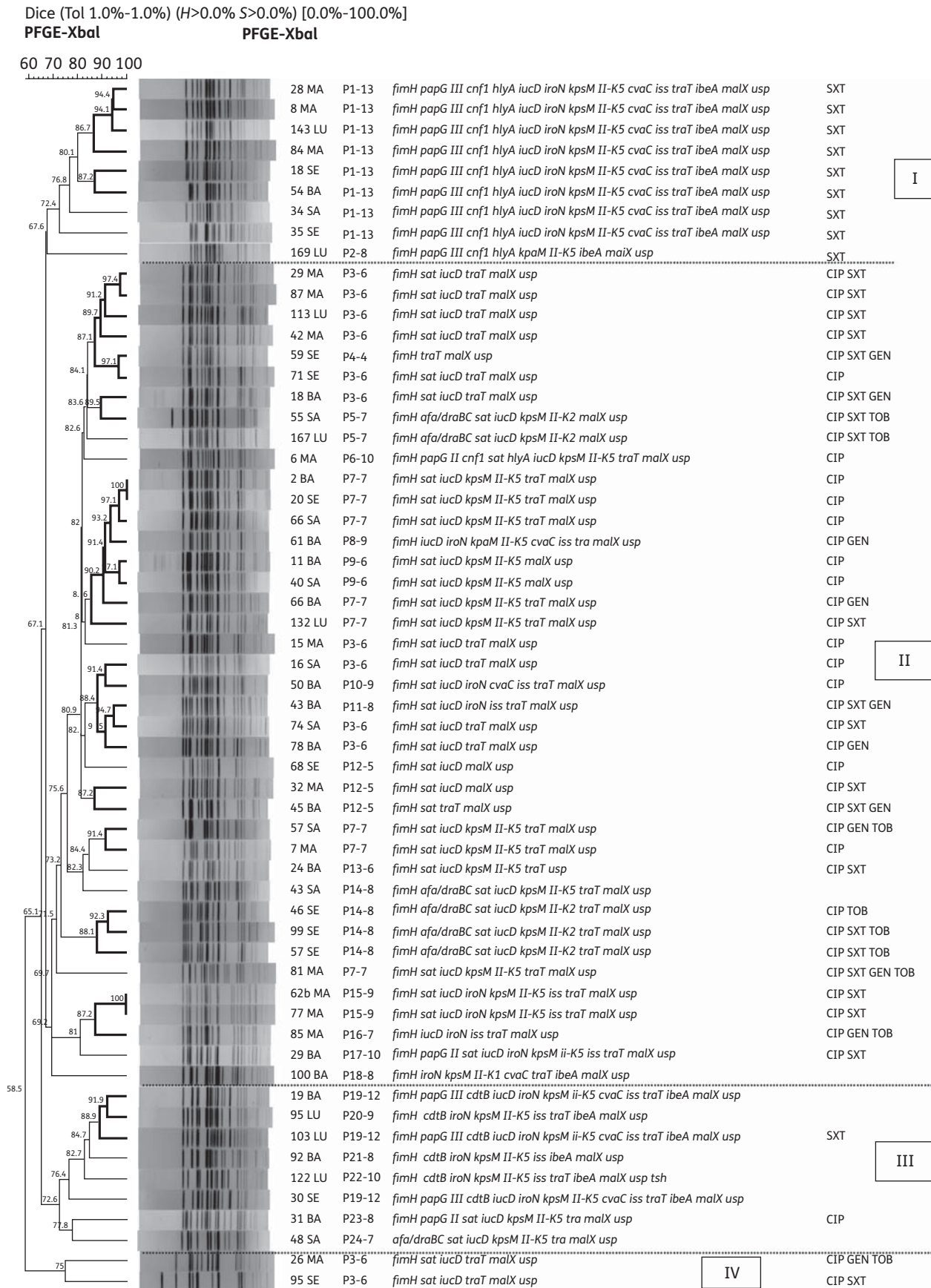
Virulence genes	Clonal groups, number of isolates (%)			P value ^a		
	O25b:H4-B2-ST131 (n=59)	O15:H1-D-ST393 (n=16)	CGA-D-ST69 (n=22)	ST131 versus ST393	ST131 versus ST69	ST393 versus ST69
Adhesins						
<i>fimH</i>	58 (98%)	16 (100%)	22 (100%)			
<i>fimAV_{MT78}</i>	0	16 (100%)	0	<0.001		<0.001
<i>papEF</i>	15 (25%)	14 (88%)	12 (55%)	<0.001	0.011	0.029
<i>papG I</i>	0	0	0			
<i>papG II</i>	3 (5%)	14 (88%)	8 (36%)	<0.001	0.005	<0.001
<i>papG III</i>	12 (20%)	0	0	0.043	0.016	
<i>papG IV</i>	0	0	3 (14%)		0.018	
<i>sfa/focDE</i>	0	0	0			
<i>afa/draBC</i>	7 (12%)	0	1 (5%)			
<i>afa</i> FM955459	4 (7%)	0	0			
<i>bmaE</i>	0	0	1 (5%)			
<i>gafD</i>	0	0	1 (5%)			
Toxins						
<i>cnf1</i>	10 (17%)	0	0		0.033	
<i>cdtB</i>	6 (10%)	0	0			
<i>sat</i>	39 (66%)	15 (94%)	11 (50%)	<0.001		0.004
<i>hlyA</i>	10 (17%)	0	0		0.033	
Siderophores						
<i>iucD</i>	52 (88%)	15 (94%)	19 (86%)			
<i>iroN</i>	22 (37%)	0	5 (23%)	0.002		
Capsula						
<i>kpsM II</i>	43 (73%)	16 (100%)	11 (50%)	0.013	0.034	0.001
<i>kpsM II-K2</i>	6 (10%)	0	1 (5%)			
<i>kpsM II-K5</i>	36 (61%)	16 (100%)	10 (45%)	0.001		<0.001
<i>neuC-K1</i>	1 (2%)	0	0			
<i>kpsM III</i>	0	0	6 (27%)		<0.001	0.027
Miscellaneous						
<i>cvaC</i>	14 (24%)	0	5 (23%)	0.024		
<i>iss</i>	21 (36%)	0	5 (23%)	0.002		
<i>traT</i>	51 (86%)	3 (19%)	19 (86%)	<0.001		<0.001
<i>ibeA</i>	16 (27%)	0	0	0.013	0.003	
<i>malX</i> (PAI)	58 (98%)	0	0	<0.001	<0.001	
<i>usp</i>	59 (100%)	0	0	<0.001	<0.001	
<i>tsh</i>	1 (2%)	0	2 (9%)			
ExPEC status	38 (64%)	16 (100%)	13 (59%)	0.002		0.003
Strains with ≥8 virulence genes	25 (42%)	0	1 (5%)	<0.001	<0.001	

PAI, pathogenicity island.

^aP values by Fisher's exact test are shown where $P < 0.05$.

and O15:H1-D-ST393 (10%) clonal groups were concentrated within the trimethoprim/sulfamethoxazole-resistant and ciprofloxacin-resistant group. Notably, clonal group ST131 was the most prevalent in all resistance subgroups and accounted for 23% of multidrug-resistant isolates. Similar results were found by Johnson *et al.*⁶ in the Canadian study (2002–04). In another study, Johnson *et al.*⁸ estimated that clonal group ST131 accounted for >40% of all isolates exhibiting any resistance,

Figure 1. PFGE of XbaI-digested DNA from the 59 O25b:H4-B2-ST131 isolates included in this study. Clusters with ≥85% similarity are indicated in bold. Isolate designation, virulence profile designation-number of virulence genes, virulence genes and associated resistances are shown on the right. Isolates 55SA, 167LU, 6MA, 99SE, 85MA and 29BA of Group II were CTX-M-15 producing. CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; TOB, tobramycin.



>50% of trimethoprim/sulfamethoxazole- plus ciprofloxacin-resistant, aminoglycoside-resistant and multidrug-resistant isolates, and 67%–69% of extended-spectrum cephalosporin-resistant and fluoroquinolone-resistant isolates from patients hospitalized across the USA in 2007. Therefore, although the ST131 clonal group is highly prevalent among Spanish resistant isolates, its frequency in 2009 was significantly lower than that estimated in the USA during 2007. In the US study, however, 73% of isolates were obtained from blood cultures, while in our study blood isolates represented only 5% of the isolates studied. In view of our results, we cannot discount the existence of other successful clonal groups in Spain.

In the present study, the prevalence of ESBL-producing isolates was 7% (35 of 500 isolates). This prevalence is higher than that observed in a multicentre study performed in 2006 in Spain (4%).²¹ Six (10%) of the 59 ST131 isolates were positive for CTX-M-15 (55SA, 167LU, 6MA, 99SE, 85MA and 29BA isolates) and one (6%) of the 16 ST393 isolates was positive for CTX-M-14 (114LU isolate), whereas none of the 22 ST69 isolates produced ESBL enzymes. Thus, in Spain in 2009, the ST131 clone occurred frequently as a fluoroquinolone-resistant but cephalosporin-susceptible pathogen. Cagnacci *et al.*⁴ characterized 148 *E. coli* isolates displaying reduced susceptibility to ciprofloxacin and causing uncomplicated UTIs in eight European countries during 2003 to 2006. About one-third (51 isolates) belonged to two clonal groups (ST131 and ST393), but only 12 (6%) were ESBL positive.

Virulence factors

ExPEC strains have specialized virulence factors that enable them to colonize host surfaces, injure host tissues and avoid host defence systems. The 97 isolates belonging to the clonal groups (O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69) of the present study were analysed by PCR for the presence of 30 genes encoding virulence factors typical of ExPEC that cause UTIs, sepsis and meningitis. Comparisons of the virulence gene prevalence values among the three clonal groups identified significant differences (Table 3).

The virulence gene profiles identified in each clonal group were different, with 24 profiles (P1–P24) among the 59 ST131 isolates (Figure 1), 4 profiles (P25–P28) among the 16 ST393 isolates (Figure 2) and 16 profiles (P29–P44) among the 22 ST69 isolates (Figure 3). However, isolates belonging to the same clonal groups showed similar virulence gene profiles. Among the 28 ESBL-producing isolates not belonging to the three clonal groups, 18 different virulence profiles (P45–P62) were identified (Figure 4) and only 4 isolates showed virulence profiles (P25, P35 and P36) previously observed.

Notably, the ST131 isolates exhibited a significantly higher virulence score (mean/median/range 8.1/7/4–13) compared with the ST393 (6.0/6/3–7; $P=0.01$), ST69 (5.4/6/2–9; $P<0.001$) and ESBL-producing isolates belonging to other clonal groups (4.0/4/1–7; $P<0.001$). In particular, the ST131 isolates carrying the *ibeA* gene showed the highest virulence score (11.4/13/8–13). In contrast, Johnson *et al.*⁶ studying Canadian *E. coli* isolates from UTIs (2002–04) observed that the virulence scores differed only slightly among the three clonal groups. In the present study, 16 (27%) of 59 ST131 isolates carried the *ibeA* gene associated with a high virulence score, whereas in the Canadian study only 1 (2%) of 46 ST131 was positive for

the *ibeA* gene. This could explain the differences observed in both studies with respect to the virulence scores.

Sixty-seven (69%) of the 97 isolates belonging to the three clonal groups analysed in this study satisfied the criteria for ExPEC status according to the definition of Johnson *et al.*³⁴ Thus, the prevalence of ExPEC status was higher within the three clonal groups (69%) than within the 28 ESBL-producing isolates not belonging to them (14%) ($P<0.001$).

Macrorestriction profiles by PFGE

Figure 1 shows a dendrogram with the XbaI macrorestriction profiles obtained by PFGE of the 59 ST131 isolates with a similarity of 58.5%. The strains remained distributed in four groups (I–IV) having identities of 67.6%, 71.5%, 72.6% and 75.0%, respectively, characteristically defined by the virulence and resistance profiles of their isolates. Among the 59 ST131 isolates, a total of 11 clusters with $\geq 85\%$ similarity were observed. Interestingly, two of these clusters included CTX-M-15-positive and -negative isolates. Previously, Nicolas-Chanoine *et al.*¹ studied the virulence genotypes of 36 CTX-M-15-producing ST131 isolates from eight countries and three continents, and found similar pathotypes to those observed in the present study among the ST131 isolates of Group II.

The novelty of our findings is shown by the eight ST131 *ibeA* isolates of Group I, all of them with identical high virulence gene content and co-trimoxazole resistance. These eight isolates were obtained from each of the five hospitals included in the study. The virulence profile of this group had been previously described only in seven ESBL-producing strains isolated in the hospital of Barcelona in 2008¹² and one human ST131 strain non-ESBL producer isolated in the hospital of Lugo in 2009.¹¹ Comparing the PFGE profiles of those strains with the eight strains of the present study (Group I), the seven ESBL-producing strains from Barcelona formed a cluster having 88.5% similarity with three ST131 strain non-ESBL producers of Group I (28MA, 8MA and 143LU) and clustered with 82.1% similarity with three other isolates of Group I (18SE, 54BA and 84MA) (dendrogram not shown). Thus, we report for first time the spread of a new variant of the ST131 clonal group with high virulence content and that is non-ESBL-producing but co-trimoxazole resistant. The similarity of the PFGE profiles and the same virulence profile would be indicative of recent emergence. In fact, among a collection of 28 non-ESBL-producing ST131 strains with the *ibeA* gene obtained from 2464 *E. coli* blood cultures of patients admitted to the Hospital Lucus Augusti (previously named Complejo Hospitalario Xeral-Calde) from 1998 to 2009 (prevalence of 1.1%),¹¹ only one strain isolated in 2009 showed the same virulence profile as the eight ST131 isolates of Group I from the present study.

Figure 2 shows a dendrogram obtained by PFGE of the 16 O15:H1-D-ST393 isolates identified in the present study that showed a similarity of 72.2%, with a cluster of $\geq 85\%$ similarity that includes 13 isolates. Comparing the PFGE profiles of these 16 isolates with seven CTX-M-14-producing strains of this clonal group isolated in Spanish hospitals between 2005 and 2008,^{12,16} five of the seven CTX-M-14-producing strains formed a cluster having 85.8% similarity with the 13 isolates of the present study (dendrogram not shown). The virulence profiles of this clonal group

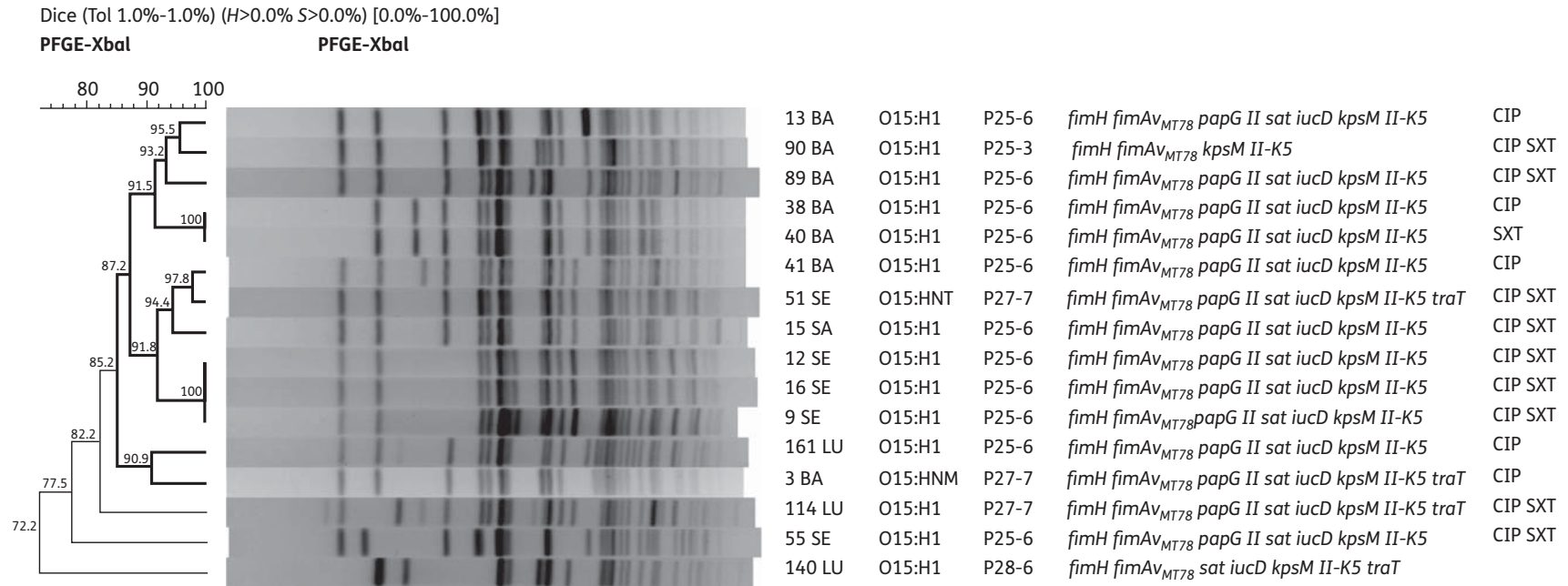


Figure 2. PFGE of XbaI-digested DNA from the 16 O15:H1-D-ST393 isolates included in this study. Clusters with $\geq 85\%$ similarity are indicated in bold. Isolate designation, serotype, virulence profile designation-number of virulence genes, virulence genes and associated resistances are shown on the right. Isolate 114LU was positive for CTX-M-14. CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole.

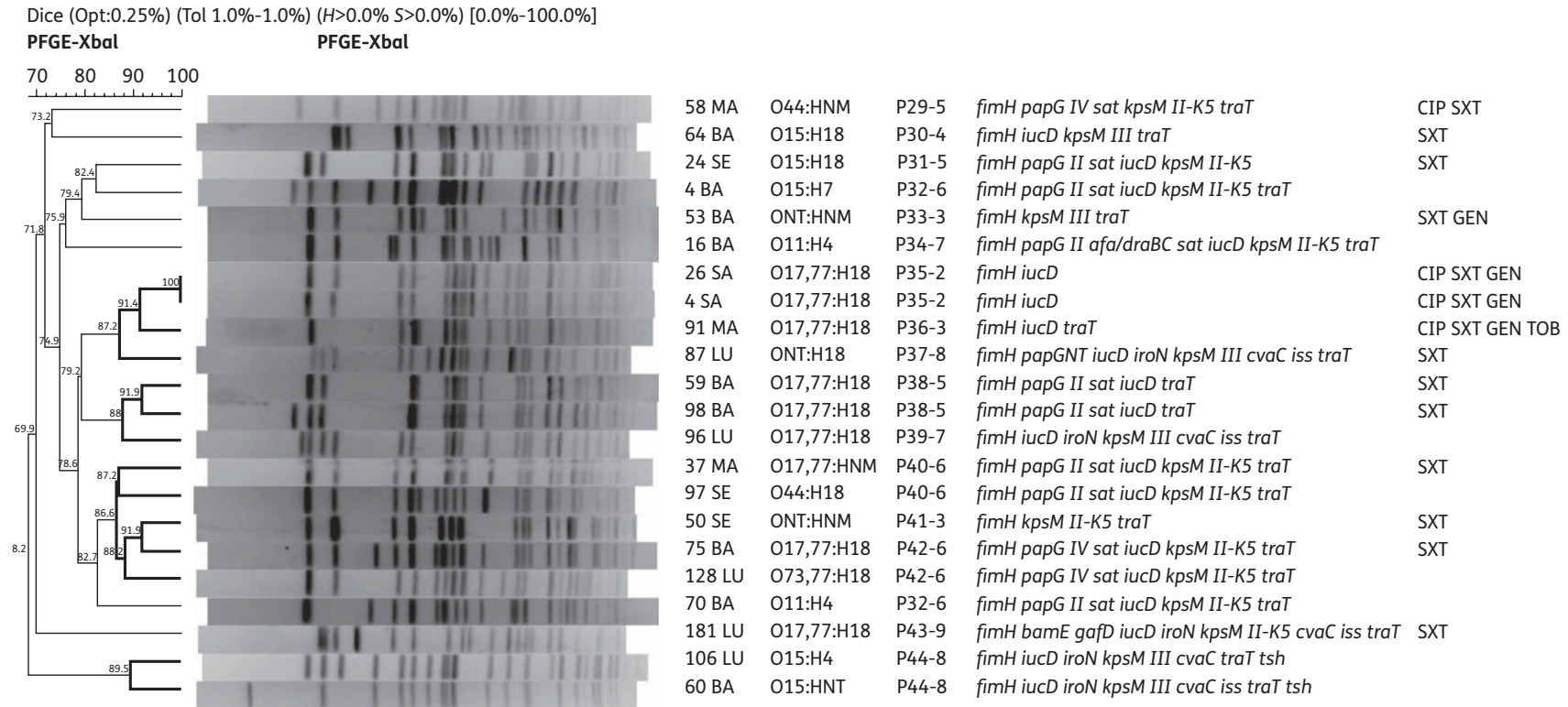


Figure 3. PFGE of XbaI-digested DNA from the 22 CGA-D-ST69 isolates included in this study. Clusters with $\geq 85\%$ similarity are indicated in bold. Isolate designation, serotype, virulence profile designation-number of virulence genes, virulence genes and associated resistances are shown on the right. CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; TOB, tobramycin.

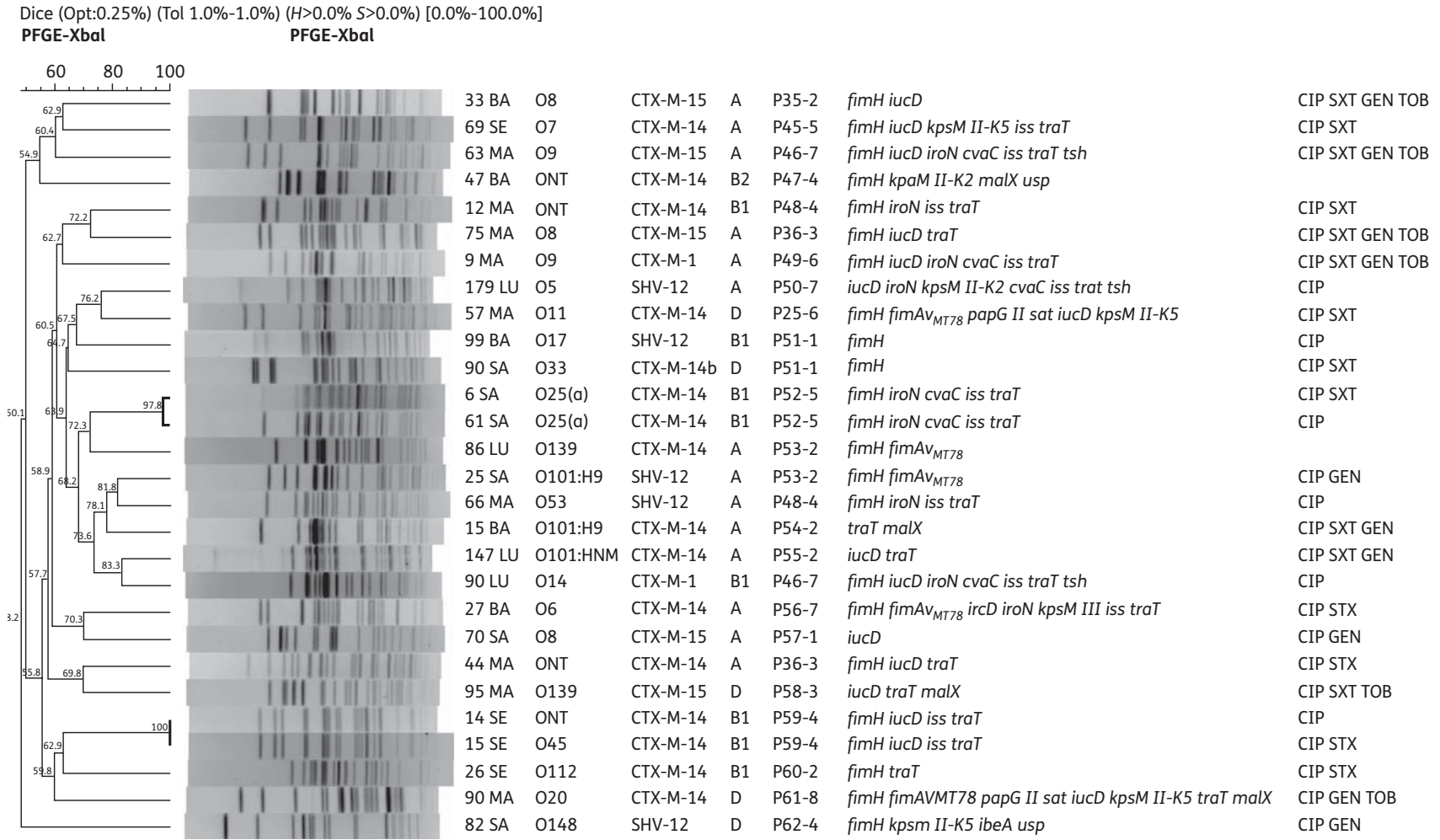


Figure 4. PFGE of XbaI-digested DNA from the 28 ESBL-producing isolates not belonging to clonal groups O25b:H4-B2-ST131, O15:H1-D-ST393 and CGA-D-ST69. Clusters with $\geq 85\%$ similarity are indicated in bold. Isolate designation, serotype, type of ESBL enzyme produced, phylogenetic groups, virulence profile designation-number of virulence genes, virulence genes and associated resistances are shown on the right. CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; TOB, tobramycin.

were very similar, including the *fimH*, *fimAv*_{MT78}, *pagG II*, *sat*, *iucD* and *kpsM II-K5* genes.

Figure 3 shows a dendrogram with the profiles obtained by PFGE of the 22 isolates belonging to CGA-D-ST69, which showed a similarity of 68.2%, with four clusters having $\geq 85\%$ similarity.

In contrast, the 28 ESBL-producing isolates not belonging to any of the three clonal groups showed 48.2% similarity, with only two clusters of $\geq 85\%$ similarity (Figure 4).

Conclusions

The three clonal groups investigated in this study accounted for 37% of isolates exhibiting trimethoprim/sulfamethoxazole plus ciprofloxacin resistance, 34% of aminoglycoside-resistant isolates and 30% of multidrug-resistant isolates, which gives evidence of an important clonal component in the emergence of resistance among ExPEC. Notably, a single high virulent clonal group (O25b:H4-B2-ST131) causes approximately 1 in every 10 extraintestinal infections in Spain. The ST131 clonal group was significantly more prevalent than the other two clonal groups characterized in this study, and exhibited a significantly higher virulence score compared with the ST393 and ST69 isolates. This is consistent with its high virulence potential in a mouse model of septicaemia³⁵ and perhaps partly explains the higher epidemiological success of the ST131 clonal group. Interestingly, a new variant of the ST131 clonal group, which is non-ESBL-producing but trimethoprim/sulfamethoxazole resistant and with high virulence content, is reported.

The high prevalence of the ST131 clonal group, particularly among multidrug-resistant isolates, has important clinical and public health implications, due to the real risk of treatment failure. Especially problematic is the dual fluoroquinolone and trimethoprim/sulfamethoxazole resistance (36% of the ST131 isolates), and these drugs constitute the empirical oral UTI therapy. Hence, alternative prophylactic strategies, such as the development of vaccines against successful clonal groups, are urgently needed.

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Transparency declarations

None to declare.

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