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Detection of quinolone-resistant *Escherichia coli* isolates belonging to clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in raw sewage and river water in Barcelona, Spain

Marta Colomer-Lluch[1]†, Azucena Mora[2]†, Cecilia López[2], Rosalia Mamani[2], Ghizlane Dahbi[2], Juan Marzoa[2], Alexandra Herrera[2], Susana Viso[2], Jesús E. Blanco[2], Miguel Blanco[2], María Pilar Alonso[3], Joan Jofre[1], Maite Muniesa[1] and Jorge Blanco[2]*

[1]*Department of Microbiology, Faculty of Biology, University of Barcelona, Barcelona, Spain;* [2]*E. coli Reference Laboratory (LREC), Department of Microbiology and Parasitology, Faculty of Veterinary Science, University of Santiago de Compostela, Lugo, Spain;* [3]*Unit of Microbiology, Lucus Augusti Hospital, Lugo, Spain*

*Corresponding author. Tel: +34-982822108; Fax: +34-982822001; E-mail: jorge.blanco@usc.es
†These authors made equal contributions to this study.

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Objectives: The present study was carried out to evaluate the prevalence of clonal group O25b:H4-B2-ST131 in water environments with faecal pollution (urban sewage and river water) in the north-east of Spain and to study the virulence gene content of environmental isolates and to compare them with isolates causing human extraintestinal infections in Spain.

Methods: This study was performed with 10 sewage samples (collected in Catalonia, north-eastern Spain, in autumn 2009 from the influent raw urban sewage of a wastewater treatment plant that serves a large urban area) and 6 river water samples (collected monthly from February to April 2010 in the Llobregat river catchment area, near Barcelona, a watercourse subjected to heavy anthropogenic pressure). *Escherichia coli* colonies were screened by PCR for the *rfbO25b* gene associated with the clonal group O25b:H4-B2-ST131. Sequence types (STs), serotypes, virulence genes, PFGE profiles, antimicrobial resistance and extended-spectrum β-lactamase (ESBL) enzymes were determined in 75 *E. coli* isolates positive for the O25b molecular subtype.

Results: Of the 75 O25b-positive isolates, 51 belonged to the O25b:H4-B2-ST131 clonal group and the remaining 24 belonged to clonal group O25b:H4-D-ST69. The majority of ST69 isolates (23 of 24) were isolated from urban sewage, whereas ST131 isolates were isolated from urban sewage (25 isolates) as well as from river water (26 isolates). ST131 and ST69 isolates carried 4–13 virulence genes, the majority (82%) being quinolone resistant.

Conclusions: We showed the presence of *E. coli* isolates belonging to clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in raw sewage and river water in Barcelona. Furthermore, we observed that the environmental O25b:H4-B2-ST131 isolates showed similar virulence and macrorestriction profiles to clinical human isolates. To our knowledge, this is the first study describing the O25b:H4-D-ST69 clonal group.

Keywords: CTX-M-15, *E. coli*, ESBLs, virulence genes

Introduction

Recently, an intercontinental *Escherichia coli* clonal group producing CTX-M-15 with a high virulence potential has been reported and represents a major public health concern.[1–3] This clonal group belongs to the B2 phylogenetic group, to the serotype O25b:H4 and to the multilocus sequence type ST131 (where ST stands for sequence type). 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. This clonal group is commonly associated with the dissemination of fluoroquinolone resistance and only some (<10%) isolates produce extended-spectrum β-lactamases (ESBLs).[4–9] The O25b:H4-B2-ST131 isolates possess numerous virulence determinants, being the causative agents of 12% of extraintestinal infections in humans and accounting for 23% of multidrug-resistant extraintestinal pathogenic *E. coli* (ExPEC) isolates in Spain.[10,11]

Currently, it is assumed that O25b:H4-B2-ST131 isolates circulate not only among humans but also among animal hosts. Thus, this clonal group has been recently identified as a cause of clinical infections in companion animals and poultry.[12–14]

The reasons underlying the successful dissemination of O25b:H4-B2-ST131 are unknown, as is the extent to which it has contaminated the wider environment. Nevertheless, it is clear that faecal pollution can contribute to the spread of this clone in the environment and could play a role in its dissemination. The global occurrence of antibiotic resistance genes in bacteria in water environments is an increasing concern, and there are many reports of microorganisms that carry genes encoding resistance to a broad range of antibiotics found in hospital wastewater and animal production wastewater as well as in urban sewage, wastewater treatment plant (WWTP) effluents, surface water, river water, groundwater and drinking water.[15,16] There is, however, scarce information about clone O25b:H4-B2-ST131. Recent reports from the UK[17] and the Czech Republic[18] indicate that isolates from the clonal group O25b:H4-B2-ST131 can be isolated from urban sewage and river water, but the virulence genotypes were not reported and the environmental isolates were not compared with human ST131 ExPEC isolates. Furthermore, there are no reports about the environmental prevalence of this clone in Spain. For these reasons, the present study was carried out to evaluate the prevalence of clonal group O25b:H4-B2-ST131 in environmental waters with faecal pollution (urban sewage and river water) in the north-east of Spain. Another aim was to characterize and compare the virulence gene content of environmental isolates belonging to ST131 and to other clonal groups detected in this study with isolates causing human extraintestinal infections in Spain.

Materials and methods

Samples

This study was performed with 10 sewage samples collected in Catalonia, north-eastern Spain, in autumn 2009, from the influent raw urban sewage of a WWTP that serves a large urban area consisting of a number of cities and towns of ~1000000 inhabitants. In addition, six samples were collected monthly from February to April 2010 in the Llobregat river catchment area, near Barcelona, a watercourse subjected to heavy anthropogenic pressure. The scarce and irregular contribution of animal faecal contaminants has its origin in husbandry activities in the upper course and occasional wild birds in the lower course.[19] The river samples were selected because the water there consistently contains numbers of faecal coliform bacteria ~3–4 orders of magnitude lower than the numbers in sewage, and will allow evaluation of the prevalence of the clone in samples containing recent pollution but are not so heavily polluted as raw sewage.[19] All samples were collected in sterile containers, transported to the laboratory at 5±2°C within 2 h of collection and processed immediately for enumeration of bacteria.

The *E. coli* isolates of this study were compared with 59 O25b:H4-B2-ST131 and 22 CGA-D-ST69 previously characterized *E. coli* isolates causing human extraintestinal infections in Spain.[11]

Microbiological parameters and bacterial isolation

Aerobic bacteria and *E. coli* present in the samples were evaluated by a membrane filtration method as standardized previously.[20] Briefly, decimal serial dilutions of urban sewage and river water in PBS were filtered through 0.45 μm pore-size membranes (Millipore, Mosheim, France), and the membranes were placed on the corresponding agar media. Aerobic bacteria were grown in Trypticase soy agar (TSA), *E. coli* enumeration was performed on Chromocult® coliform agar (Merck, Darmstadt, Germany), and plates were incubated in aerobic conditions at 37°C for 18 h.

To evaluate the presence of aerobic bacteria and *E. coli* isolates showing resistance to ampicillin, samples were processed as described above and incubated in TSA or in Chromocult® coliform agar for 2 h at 37°C. Then, membranes were transferred to TSA or Chromocult® coliform agar containing 32 mg/L ampicillin (Sigma-Aldrich, Steinheim, Germany) and further incubated at 37°C for 18 h. The ampicillin concentration used reflected the breakpoint concentration for testing with *E. coli*, and was previously reported for isolation of bacteria from the environment.[20,21]

For *E. coli* isolation, 20–50 blue colonies of each sample suspected to be *E. coli* were isolated from Chromocult® coliform agar plates containing ampicillin. Indole-positive *E. coli* colonies were screened by PCR for the *rfbO25b* gene associated with the clonal group O25b:H4-B2-ST131.[22] In total, 662 colonies were screened (390 colonies from 10 samples of raw sewage and 272 colonies from 6 samples of Llobregat river). The 75 *E. coli* isolates positive for the O25b molecular subtype were identified as *E. coli* using the MicroScan WalkAway automated system (Siemens Healthcare Diagnostics Ltd).

Bacterial DNA isolation and standard PCR procedures

Each colony was resuspended in 25 μL of double-distilled sterile water, heat treated in a bath at 95°C for 10 min and immediately transferred to ice-cold absolute ethanol. Samples were then centrifuged at 16000 **g** for 5 min and 2 μL of the supernatant was used as a template for PCR amplification. PCR amplifications were performed with a GeneAmp PCR System 2400 (Perkin-Elmer, PE Applied Biosystems, Barcelona, Spain). Molecular subtype O25b was identified based on a recently described molecular approach, which amplifies a 300 bp fragment with primers rfb.1bis and rfbO25b.r.[5,22] Afterwards, the isolates were confirmed to be O25b:H4 by serotyping.

O and H typing

Determination of O and H antigens was carried out using the method previously described by Guinée *et al.*[23] with all available O (O1–O181) and H (H1–H56) antisera. Additionally, the specific O25a and O25b molecular subtypes and the flagellar H4 gene (*fliC*-H4) were determined by PCR using oligonucleotide primers described elsewhere.[22,24]

Phylogenetic grouping and multilocus sequence typing (MLST)

Isolates were assigned to one of the four main phylogenetic groups of *E. coli* (A, B1, B2, D) by using the multiplex PCR-based method of Clermont *et al.*[25] MLST was achieved as previously described 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 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.[26]

Antimicrobial susceptibility and ESBL typing

Susceptibility to antibiotics was analysed by broth microdilution. Minimal inhibitory concentrations were determined using a MicroScan WalkAway automated system according to the manufacturer's instructions. Intermediate susceptibility was not considered as resistant. Resistance was interpreted based on the recommended breakpoints of the CLSI.[27]

Suggestive evidence of ESBL production was defined as synergy between amoxicillin/clavulanate and cefotaxime or ceftazidime. To determine the genotype of ESBLs, PCR and sequencing were performed using the TEM-, SHV-, CTX-M-1 and CTX-M-9 group-specific primers as reported previously.[28]

Virulence genotyping

The presence of 30 virulence genes was analysed as documented previously,[10,11,29–31] using primers specific for genes and operons that encode extraintestinal virulence factors (VFs) characteristic of ExPEC: *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

PFGE analysis with XbaI digestion was performed as described previously.[29] The PFGE profiles were analysed with the BioNumerics fingerprinting software (Applied Maths, St-Martens-Latem, Belgium). Cluster analysis of Dice similarity indices based on the unweighted pair group method using arithmetic linkages (UPGMA) was done to generate a dendrogram describing the relationship among the PFGE profiles. Isolates were considered related if their Dice similarity index was >85%, according to the criterion of Tenover *et al.*[32] (a difference of six bands or less).

Statistical analysis

Comparisons of proportions were tested using Fisher's exact test. For each comparison, a *P* value of <0.05 was considered to denote a significant difference.

Results and discussion

Prevalence of aerobic bacteria and E. coli in water samples

Results showed values of total aerobic bacteria averaging 6 $\log_{10}$ units of aerobic bacteria cfu and 4 $\log_{10}$ units of *E. coli* cfu per mL of sewage (Table 1). Among these, most isolates showed resistance to ampicillin (32 mg/L), and the densities of ampicillin-resistant colonies detected were <1 $\log_{10}$ units cfu/mL below the densities obtained without ampicillin. River water samples showed densities of both parameters 3 $\log_{10}$ units less than urban sewage water samples. These values were in accordance with recent reports of samples from the same origin taken on different dates.[33]

Human and animal pathogenic and potentially pathogenic bacteria are constantly released with wastewater into the water environment. Many characteristics of faecal polluted water make it a highly suspect medium for the spread of antibiotic resistance genes, i.e. the presence of antibacterials from household products (soaps, detergents, etc.), the presence of antibiotics that have been excreted by humans or disposed of down the drain, and a high bacterial load. An important part of the dispersal and evolution of antibiotic-resistant bacterial organisms depends on water environments. In water, bacteria of different origins (human, animal and environmental) are able to mix, and resistance evolves as a consequence of promiscuous exchange and shuffling of genes, genetic platforms and genetic vectors.[15]

Prevalence of clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in water samples

Characteristic β-glucuronidase-positive *E. coli* colonies (presenting a blue colour) grown on the Chromocult® coliform agar plates were randomly selected and tested by PCR for the *rfbO25b* gene, and 20–50 colonies per sample were subcultured. All colonies were further confirmed as *E. coli* by the indole test, as recommended by the manufacturer (Merck). In 15 of the 16 samples analysed, at least one positive isolate of molecular subtype O25b was obtained. In total, 75 positive O25b isolates were identified (Table 1). The averaged prevalence of O25b isolates was calculated for each sample [(number of O25b isolates/total number of *E. coli* isolates in the sample) ×100]. The average for all samples was 12.3% in raw sewage and 9.9% in river water.

Of the 75 O25b-positive isolates, 51 belonged to the O25b:H4-B2-ST131 clonal group while the remaining 24 belonged to O25b:H4-D-ST69 (Table 2). The majority of ST69 isolates were isolated from urban sewage (23 of 24 isolates from 4 of 10 samples), whereas 25 ST131 isolates were isolated from urban sewage (from 9 of 10 samples) and 26 from river water (from 6 of 6 samples) (Table 1). It should be remembered that, as for urban sewage, isolates from Llobregat river were most likely of human origin, since there is a very low possibility of animal faecal contamination in the lower course of this river.

In the present study, we showed that ST69 isolates belonged to serotype O25b:H4 by using serotyping and PCR typing (O25b rfb variant and *fliC*-H4). Thus, the serotype O25b:H4 usually associated with B2-ST131 isolates can also be found in isolates belonging to other clonal groups. Previously, other examples have been reported in this line, e.g. isolates of serotype O1:K1:H7 belonging to clonal groups B2-ST95 and D-ST59[29] and isolates of serotype O2:H6 belonging to clonal groups B2-ST998 and D-ST115.[34]

Quinolone resistance and ESBL production

The majority (75%) of 51 ST131 and all 24 ST69 isolates were resistant to nalidixic acid and a significant proportion (61%) of ST131 isolates were also fluoroquinolone resistant, while no ST69 isolates were fluoroquinolone resistant. However, ESBL production was detected in only 6 (12%) of the 51 O25b:H4-B2-ST131 isolates (5 CTX-M-15-producing isolates from three raw sewage samples and 1 CTX-M-1-producing isolate from river water). All 24 isolates belonging to the clonal group O25b:H4-D-ST69 were negative for ESBL production. This prevalence is similar to that observed in a Spanish national survey of *E. coli* causing extraintestinal infections in humans performed in 2009.[11] In that study, 6 (10%) of the 59 human ST131 isolates were positive for CTX-M-15, whereas none of the 22 human ST69 isolates produced ESBL enzymes. Thus, the Spanish environmental and human O25b:H4-B2-ST131 clonal group occurs frequently as a quinolone-resistant but cephalosporin-susceptible pathogen.

Dhanji *et al.*[17] sampled River Thames water from three urban sites (City of London) on two occasions (1 week apart, 23 and 30 March 2010) and recovered O25b:H4-B2-ST131 *E. coli* isolates

Table 1. Microbiological analysis of the water samples and prevalence of clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69

	Aerobic bacteria (cfu/mL)	Aerobic bacteria + 32 mg/L AMP (cfu/mL)	*E. coli* (cfu/mL)	*E. coli* + 32 mg/L AMP (cfu/mL)	O25b isolates[a]	O25b:H4-B2-ST131[b]	O25b:H4-D-ST69[b]
Urban sewage (n=10)							
average	3.7×10^6	1.9×10^6	5.7×10^4	1.9×10^4	48	25	23
max	9.4×10^6	3.9×10^6	2.8×10^5	7.9×10^4		-	-
min	1.8×10^6	5.5×10^5	1.6×10^4	5.0×10^3		-	-
River water (n=6)							
average	7.0×10^3	2.4×10^3	4.0×10^1	1.5×10^1	27	26	1
max	9.9×10^3	1.4×10^4	1.4×10^2	8.5×10^1		-	-
min	8.7×10^2	2.0×10^2	2.5×10^0	1.4×10^0		-	-

AMP, ampicillin.
[a]Number of O25b-positive isolates among colonies selected randomly from the ampicillin-resistant *E. coli* colonies.
[b]Number of isolates of ST131 and ST69 among the 75 O25b isolates.

only on 23 March. This study showed that, among a total of 30 ciprofloxacin-resistant isolates, 20 belonged to the O25b:H4-B2-ST131 clonal group. Ten of 20 ST131 isolates harboured CTX-M-14 enzyme but none produced CTX-M-15, which is the most common ESBL among human clinical ST131 isolates.

Similarly, Dolejska *et al.*[18] detected O25b:H4-B2-ST131 isolates in 12 (27%) of 45 wastewater samples taken daily between November 2008 and February 2009 from the outflow of a municipal WWTP in Brno city (Czech Republic). Water samples were cultivated on MacConkey agar with cefotaxime and 17 of 19 O25b:H4-B2-ST131 isolates were positive for CTX-M-15 enzyme.

To our knowledge, this is the first study to detect the O25b:H4-D-ST69 clonal group. In previous studies, the ST69 was observed in isolates of clonal group A (CGA) belonging to phylogenetic group D and showing different O serogroups with the H18 flagellar antigen. CGA-D-ST69 accounted for up to 50% of trimethoprim/sulfamethoxazole-resistant urinary tract infections due to *E. coli* in the USA and 4% of total human extra-intestinal infections caused by *E. coli* in Spain.[11,35] CGA isolates belonging to serotypes O11:H18, O15:H18, O17:H18, O44:H18, O17,O77:H18, O73:H18, O77:H18 and O86:H18 were present in four of seven sewage effluents collected from geographically dispersed areas of the USA.[36]

Virulence factors

ExPEC isolates typically possess diverse specialized VFs, including adhesins, toxins, iron acquisition siderophores, polysaccharide capsules and other miscellaneous factors that enable them to colonize host surfaces, injure host tissues and avoid host defence systems. The 75 isolates belonging to the clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in the present study were analysed by PCR for the presence of 30 genes encoding VFs typical of ExPEC that cause urinary tract infections, sepsis and meningitis. Of the 30 virulence genes tested, 21 were detected in at least 1 isolate each. Isolates carried 4–13 virulence genes. Comparison of the virulence gene content of the two clonal groups showed significant differences (Table 2 and Figure 1). ST131 isolates carried a significantly higher number of virulence genes (mean 8.2, range 4–13) than ST69 isolates (all showed the same four virulence genes).

A total of 62 (83%) of 75 O25b:H4 isolates from water samples in this study satisfied criteria for ExPEC status according to a modification of the criteria of Johnson *et al.*,[37] as they carried two or more of the *papEF*, *sfa/focDE*, *afa/draBC*, *iucD* and *kpsM II* genes.

As in previous studies,[10–12] O25b:H4-B2-ST131 isolates carrying the *ibeA* gene (invasion of brain endothelium) in the present study exhibited a higher virulence gene content than *ibeA*− isolates. Thus, 100% (18 of 18) of the *ibeA*+ isolates carried eight or more virulence genes versus 6% (2 of 33) of the O25b:H4-B2-ST131 *ibeA*− isolates ($P<0.001$). On the contrary, none of the O25b:H4-D-ST69 isolates was positive for the *ibeA* gene ($P<0.001$) and none carried more than four virulence genes (Table 2).

Thirteen (72%) of 18 O25b:H4-B2-ST131 *ibeA*+ isolates were recovered from river water versus 5 (28%) from urban sewage ($P=0.02$).

Interestingly, 31 (94%) of 33 O25b:H4-B2-ST131 *ibeA*− isolates exhibited resistance to ciprofloxacin and 18 (54%) resistance to tobramycin. In contrast, none of the 18 ST131 *ibeA*+ isolates was resistant to fluoroquinolones ($P<0.001$) and only 1 (6%) isolate was resistant to tobramycin ($P<0.001$).

Previously, Nicolas-Chanoine *et al.*,[1] studying the virulence genotypes of 36 CTX-M-15-producing O25b:H4-B2-ST131 human isolates from eight countries and three continents, found similar pathotypes to those detected in the present study among O25b:H4-B2-ST131 *ibeA*− isolates, with the difference that only 5 of 51 ST131 isolates in the present study were CTX-M-15 producing.

Remarkably, the 12 O25b:H4-D-ST69 environmental isolates showed the presence of *papG* allele I and the absence of group 2 capsule genes. In contrast, none of the 22 CGA-D-ST69 isolates causing infections in humans characterized in a previous study[11] showed the *papG* allele I, and 11 of those 22 carried group 2 capsule genes (*kpsM II-K5*).

Macrorestriction profiles by PFGE: comparison with clinical human isolates

Sixty-three isolates were analysed by PFGE: 18 O25b:H4-B2-ST131 *ibeA*+ isolates, 33 O25b:H4-B2-ST131 *ibeA*− isolates and 12 of the

Table 2. Prevalence of virulence genes in environmental isolates of clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69

	Clonal groups			*P*[a]		
Virulence genes	O25b:H4-B2-ST131 *ibeA*+ (*n*=18)	O25b:H4-B2-ST131 *ibeA*− (*n*=33)	O25b:H4-D-ST69 (*n*=24)	ST131 *ibeA*+ versus ST131 *ibeA*−	ST131 *ibeA*+ versus ST69	ST131 *ibeA*− versus ST69
Adhesins						
fimH	18 (100%)	31 (94%)	24 (100%)			
fimAv$_{MT78}$	0	0	0			
papEF	15 (83%)	2 (6%)	24 (100%)	<0.001		<0.001
papG I	0	0	24 (100%)		<0.001	<0.001
papG II	0	2 (6%)	0			
papG III	15 (83%)	0	0	<0.001	<0.001	
papG IV	0	0	0			
sfa/focDE	0	0	0			
afa/draBC	1 (6%)	1 (3%)	0			
afaFM955459	0	0	0			
bmaE	0	0	0			
gafD	0	0	0			
Toxins						
cnf1	6 (33%)	1 (3%)	0	0.006	0.004	
cdtB	9 (50%)	0	0	<0.001	<0.001	
sat	2 (11%)	31 (94%)	0	<0.001		<0.001
hlyA	6 (33%)	1 (3%)	0	0.006	0.004	
Siderophores						
iucD	16 (89%)	31 (94%)	24 (100%)			
iroN	14 (78%)	0	0	<0.001	<0.001	
Capsules						
kpsM II	18 (100%)	20 (61%)	0	0.001	<0.001	<0.001
kpsM II-K2	0	0	0			
kpsM II-K5	17 (94%)	20 (61%)	0	0.009	<0.001	<0.001
neuC-K1	1 (6%)	0	0			
kpsM III	0	0	0			
Miscellaneous						
cvaC	14 (78%)	0	0	<0.001	<0.001	
iss	14 (78%)	0	0	<0.001	<0.001	
traT	18 (100%)	27 (82%)	24 (100%)			
ibeA	18 (100%)	0	0	<0.001	<0.001	
malX (PAI)	18 (100%)	33 (100%)	0		<0.001	<0.001
usp	18 (100%)	33 (100%)	0		<0.001	<0.001
tsh	1 (6%)	0	0			
ExPEC status	18 (100%)	20 (61%)	24 (100%)	0.001		<0.001
Isolates with at least eight virulence genes	18 (100%)	2 (6%)	0	<0.001	<0.001	

Continued

24 O25b:H4-D-ST69 isolates. Only 4 of 16 isolates obtained from sample US-8 were selected for PFGE typing since the 16 ST69 isolates showed the same virulence gene content and antibiotic resistance profile. The dendrogram derived from analysis of the macrorestriction profiles generated three different groups corresponding to isolates O25b:H4-B2-ST131 *ibeA*+ (I), O25b:H4-B2-ST131 *ibeA*− (II) and O25b:H4-D-ST69 (III), characteristically defined by their virulence profiles and resistance patterns

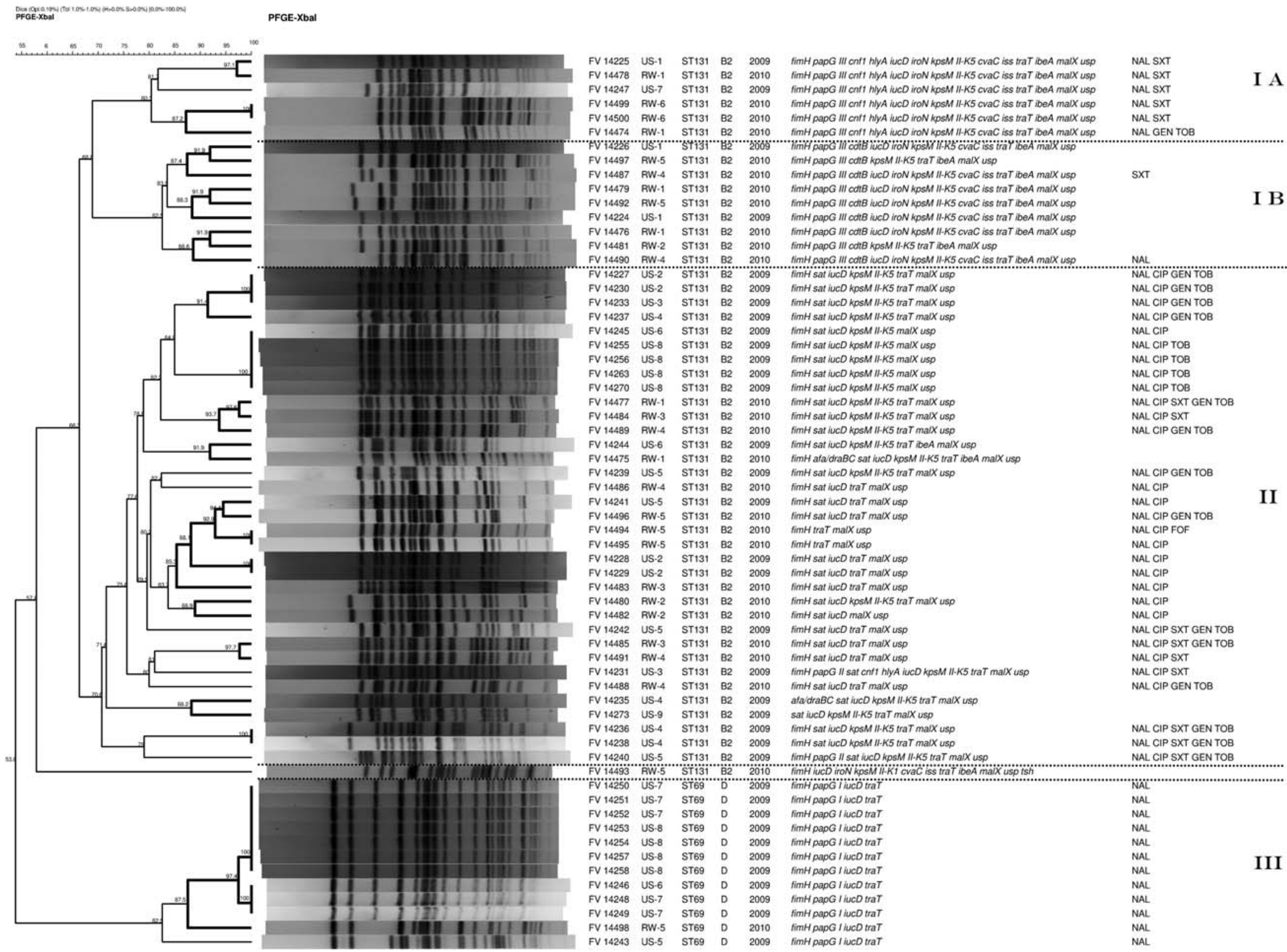

Figure 1. PFGE of XbaI-digested DNA from the 63 *E. coli* O25b:H4 isolates from urban sewage (US) and river water (RW). The dendrogram was obtained with the UPGMA algorithm based on the Dice similarity coefficient and applying 1% tolerance in the band position. Isolate designation, origin, ST, phylogenetic group, year of isolation, virulence gene profile and antibiotic resistance pattern are shown on the right. Isolates FV14227, FV14230, FV14231, FV14233 and FV14240 were positive for CTX-M-15 and isolate FV14493 was positive for CTX-M-1. NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; SXT, co-trimoxazole; FOF, fosfomycin.

(Figure 1). ST131 isolates showed a higher genetic diversity (41 pulsotypes with 14 clusters of ≥85% similarity), while the ST69 isolates showed only 4 pulsotypes with 1 cluster of ≥85% similarity grouping 11 of 12 isolates. Interestingly, ST131 isolates with different PFGE profiles and virulence gene contents were isolated from the same water sample in 11 cases (samples RW-1, RW-2, RW-3, RW-4, RW-5, US-1, US-2, US-3, US-4, US-5 and US-6).

Comparing the macrorestriction profiles, virulence genes and resistance patterns of the 51 environmental O25b:H4-B2-ST131 isolates of the present study with 59 clinical human isolates of this clonal group from a Spanish national survey,[11] we can confirm that the O25b:H4-B2-ST131 isolates from the water samples of this study showed similar virulence gene contents and macrorestriction profiles to those of human origin (Figure S1, available as Supplementary data at *JAC* Online). Among the 110 isolates, a total of 20 clusters with similarity ≥85% were observed. Interestingly, eight of these clusters included clinical human isolates and environmental isolates from both urban sewage and water samples.

In contrast, the 12 environmental isolates of clonal group O25b:H4-D-ST69 showed very different virulence gene contents, resistance patterns and PFGE profiles from the 22 CGA-D-ST69 isolates causing infections in humans[11] (Figure S2, available as Supplementary data at *JAC* Online).

Conclusions

In summary, we showed the presence of quinolone-resistant *E. coli* isolates belonging to clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in raw sewage and river water in Barcelona. Furthermore, environmental O25b:H4-B2-ST131 isolates showed similar virulence and macrorestriction profiles to clinical human isolates. To our knowledge, this is the first study to detect the O25b:H4-D-ST69 clonal group.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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