Inhibitor-Resistant TEM- and OXA-1-Producing *Escherichia coli* Isolates Resistant to Amoxicillin-Clavulanate Are More Clonal and Possess Lower Virulence Gene Content than Susceptible Clinical Isolates

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In a previous prospective multicenter study in Spain, we found that OXA-1 and inhibitor-resistant TEM (IRT) β-lactamases constitute the most common plasmid-borne mechanisms of genuine amoxicillin-clavulanate (AMC) resistance in *Escherichia coli*. In the present study, we investigated the population structure and virulence traits of clinical AMC-resistant *E. coli* strains expressing OXA-1 or IRT and compared these traits to those in a control group of clinical AMC-susceptible *E. coli* isolates. All OXA-1-producing ($n = 67$) and IRT-producing ($n = 45$) isolates were matched by geographical and temporal origin to the AMC-susceptible control set ($n = 56$). We performed multilocus sequence typing and phylogenetic group characterization for each isolate and then studied the isolates for the presence of 49 virulence factors (VFs) by PCR and sequencing. The most prevalent clone detected was distinct for each group: group C isolates of sequence type (ST) 88 (C/ST88) were the most common in OXA-1 producers, B2/ST131 isolates were the most common in IRT producers, and B2/ST73 isolates were the most common in AMC-susceptible isolates. The median numbers of isolates per ST were 3.72 in OXA-1 producers, 2.04 in IRT producers, and 1.69 in AMC-susceptible isolates; the proportions of STs represented by one unique isolate in each group were 19.4%, 31.1%, and 48.2%, respectively. The sum of all VFs detected, calculated as a virulence score, was significantly higher in AMC-susceptible isolates than OXA-1 and IRT producers (means, 12.5 versus 8.3 and 8.2, respectively). Our findings suggest that IRT- and OXA-1-producing *E. coli* isolates resistant to AMC have a different and less diverse population structure than AMC-susceptible clinical *E. coli* isolates. The AMC-susceptible population also contains more VFs than AMC-resistant isolates.

*E*scherichia coli is an important etiologic agent that causes both nosocomial and community-acquired infections (1). Amoxi-cillin-clavulanate (AMC) is a widely used antibiotic in many countries and is often used to treat *E. coli* infections (2, 3). According to the European Antimicrobial Resistance Surveillance Network (EARS-Net), the percentage of *E. coli* blood isolates in Spain that are nonsusceptible to AMC increased from 9.3% in 2003 to 25.3% in 2012 (http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/database/Pages/database.aspx). This increase in resistance coincides with a dramatic increase in the community consumption of AMC (4).

AMC resistance in *E. coli* results from a complex epidemiological background involving the clonal and nonclonal spread of several resistance mechanisms (5). The production of OXA-1 and inhibitor-resistant TEM (IRT) β-lactamases is the most common plasmid-borne mechanism of AMC resistance in *E. coli* and does not affect other broad-spectrum β-lactam antibiotics (5). Clinical data for patients infected by AMC-resistant *E. coli* have also been provided by our group (6). However, there is very little information available about the population structure and virulence-associated determinants of OXA-1- and IRT-producing *E. coli* isolates compared with the amount of information available for AMC-susceptible clinical isolates.

Most extraintestinal pathogenic *E. coli* (ExPEC) isolates belong to phylogenetic group B2 and, to a lesser extent, to group D. ExPEC strains possess high numbers of virulence factors (VFs), including toxins, adhesins, polysaccharide capsules, siderophores, and invasins, that may enable them to evade host defenses and invade host tissues. *E. coli* isolates of phylogroups A and B1 are mainly found as part of the intestinal commensal population and usually possess a lower number of VFs (7, 8). In *E. coli*, several studies have evaluated the linkage between virulence and resistance to antimicrobials, such as quinolones, trimethoprim-sulfamethoxazole, or cephalosporins. Most of these studies have shown

TABLE 1 Different population markers indicating genetic and virulence variations between OXA-1-producing-, IRT-producing, and susceptible *Escherichia coli* isolates[a]

Population and virulence marker	Result by resistance mechanism			P value[b]			
	OXA-1 production (n = 67)	IRT production (n = 45)	AMC susceptible (n = 56)	OXA-1 vs IRT	OXA-1 vs AMC susceptible	IRT vs AMC susceptible	AMC resistant vs AMC susceptible
No. (%) of isolates							
From urine	49 (73.1)	35 (77.8)	41 (73.2)	0.65	1	0.65	0.85
Invasive	11 (16.4)	1 (4.4)	10 (17.9)	0.07	1	0.07	0.22
From females	36 (53.7)	30 (66.7)	33 (58.9)	0.23	0.70	0.54	1
From patients >65 yr of age	46 (68.7)	20 (44.4)	22 (39.3)	**0.01**	**0.002**	0.69	**0.01**
No. of STs	18	22	33				
Median (range) no. of isolates per ST	3.72 (1–25)	2.04 (1–8)	1.69 (1–12)				
No. (%) of single isolates by ST	13 (19.4)	14 (31.1)	27 (48.2)	0.18	**0.009**	0.10	**0.03**
No. (%) of isolates by phylogroup							
B2	23 (34.3)	24 (53.3)	59 (69.6)	0.53	**0.0001**	0.1	**0.001**
B2/ST131	22 (32.8)	8 (17.8)	3 (5.36)	0.08	**0.0001**	0.06	**0.0008**
B2/ST73	1 (1.5)	5 (11.1)	12 (21.4)	**0.04**	**0.0005**	0.19	**0.003**
C	36 (53.7)	8 (17.8)	5 (8.9)	**0.0002**	**<0.0001**	0.23	**<0.0001**
C/ST88	25 (37.3)	0	0	**<0.0001**	**<0.0001**		**<0.0001**
Virulence score (range)	8.3 (1–13)	8.2 (2–17)	12.5 (1–19)	0.2722	**<0.0001**	**<0.0001**	**<0.0001**

[a] IRT, inhibitor resistant TEM; AMC, amoxicillin-clavulanate; ST, sequence types.
[b] Boldface data indicate statistically significant differences.

that antibiotic-susceptible *E. coli* isolates are usually more virulent than resistant ones (9–11). However, in the last few years, *E. coli* group B2 clones possessing a high number of VFs and resistance to several antimicrobials have emerged (i.e., sequence type [ST] 131 [ST131]) (12–14). According to recent data (15), eight phylogroups are now recognized: seven (A, B1, B2, C, D, E, F) belong to *E. coli sensu stricto*, whereas the eighth is *Escherichia* cryptic clade I.

Our hypothesis was that AMC, the antibiotic most consumed by far in Spain and other countries (3, 4), could select for not only AMC resistance but also certain specific clones carrying AMC resistance (16). To clarify this subject, the population structure of both resistant and susceptible isolates was studied in parallel. The objective of this study was to determine the population structure and virulence traits of clinical AMC-resistant *E. coli* isolates due to OXA-1 or IRT production in comparison with those of a control group of clinical AMC-susceptible *E. coli* isolates.

MATERIALS AND METHODS

Study design and bacterial isolates. As described previously (5), 257 nonduplicated, AMC-resistant *E. coli* isolates were collected from clinical samples at seven Spanish hospitals in six geographic regions between January and March of 2010. Of these, all 112 (43.6%) isolates producing either OXA-1 (n = 67) or IRT (n = 45) were included in this study. The IRT types were TEM-40 (n = 15), TEM-30 (n = 13), TEM-33 (n = 5), TEM-32 (n = 2), TEM-34 (n = 2), TEM-35 (n = 1), TEM-54 (n = 1), TEM-76 (n = 1), TEM-79 (n = 1), and TEM-185 (n = 4). Among the 67 OXA-1-producing isolates, 25 (37.3%) were also CTX-M-15 producers.

Additionally, 56 AMC-susceptible (MIC < 4/2 μg/ml) clinical isolates were simultaneously collected at the participant hospitals to constitute the AMC-susceptible control group. These AMC-susceptible isolates were matched by geographical and temporal origin and were susceptible to several other β-lactam antibiotics. Susceptibility to AMC and other anti-

biotics, including ampicillin, cephalosporins, carbapenems, quinolones, aminoglycosides, and co-trimoxazole, was confirmed at the central reference laboratory, as described previously (5).

In total, 168 *E. coli* isolates were included in this study: 67 OXA-1-producing isolates, 45 IRT-producing isolates, and 56 AMC-susceptible isolates. The origins of all the *E. coli* isolates included in this study are detailed in Table 1.

Molecular epidemiology, phylogenetic groups, and detection of serotypes O25b and O16. The multilocus sequence types (MLSTs) and phylogenetic groups of all 168 *E. coli* isolates were determined. MLST was performed according to the University of Warwick (Warwick Medical School, Coventry, United Kingdom) scheme for *E. coli* developed by M. Achtman (http://mlst.warwick.ac.uk). The phylogenetic relationships among the different STs obtained were established according to the eBURST program, version 3.

In addition, serotypes O25b and O16 were identified by allele-specific PCRs as described previously (17, 18).

Phylogenetic groups were determined both by the former method of Clermont et al. (19) and by the recently updated method of Clermont et al. (15).

VFs. In all 168 *E. coli* isolates, the presence of 49 virulence-associated genes, including 19 adhesins, 4 siderophores, 11 toxins, 6 capsule synthesis-associated genes, and 9 miscellaneous VF genes, was determined by multiplex PCRs using primers described previously (20–22). Virulence scores were calculated for each isolate as the sum of all VFs detected; *pap*, *sfa-foc*, and *clbB-clbN* were counted only once regardless of the number of elements or subunits identified.

Statistical analysis. Differences in the prevalence of phylogroups and sequence types between the different groups were assessed by Fisher's exact test. Associations were determined by calculation of the odds ratio (OR) with 95% confidence intervals (CIs). The null hypothesis was rejected for data with P values of <0.05. Statistical analysis was performed using GraphPad Prism, version 3.02, software (GraphPad Software, Inc.,
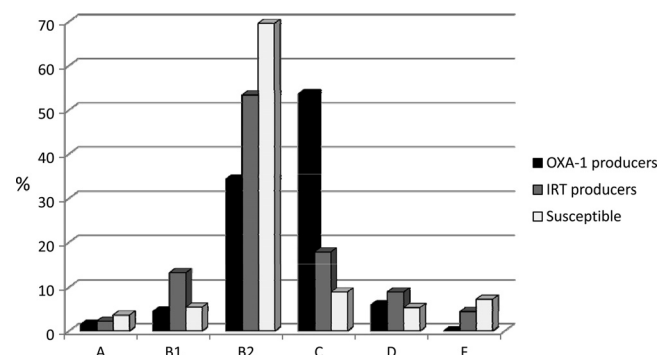
FIG 1 Distribution of phylogenetic groups among 67 OXA-1 producers, 45 IRT producers, and 56 susceptible *Escherichia coli* isolates. Phylogroup E isolates were not detected.

San Diego, CA). Virulence scores were compared by use of the Mann-Whitney U test.

RESULTS

Phylogenetic groups. The distribution of the phylogenetic groups is depicted in Fig. 1. OXA-1 producers mostly belonged to phylogroups C and B2, most IRT producers belonged to phylogroup B2, and the vast majority of susceptible isolates were of the B2 phylogroup (Table 1).

E. coli isolates producing OXA-1 but not CTX-M-15 extended-spectrum β-lactamases (ESBLs) belonged to phylogroup C, but the majority of isolates (78.3%) producing OXA-1 plus CTX-M-15 belonged to phylogroup B2.

Of the 168 *E. coli* isolates, 55 (32.7%) belonged to newly described phylogroup C (29.1%) or F (3.6%). No phylogroup E isolates were detected.

By the former method of Clermont et al. (19), 49 (29.2%) isolates were classified as phylogroup A; however, 45 of them (91.8%) were reclassified as phylogroup C.

MLST results. Eighteen different STs were identified among the 67 OXA-1-producing isolates (mean number of isolates per ST, 3.72; range, 1 to 25). Twenty-two different STs were identified among the 45 IRT-producing isolates (mean of number of isolates per ST, 2.04; range, 1 to 8), and the 56 AMC-susceptible isolates were identified to be 33 different STs (mean number of isolates per ST, 1.69; range, 1 to 12) (Tables 1 and 2). The proportions of STs represented by one unique isolate were 19.4% for the OXA-1 group, 31.1% for the IRT group, and 48.2% for the AMC-susceptible group (Table 1).

The most prevalent ST was different for each group. In the OXA-1-producing isolates, ST88 (25 isolates, 37.3%) and ST131 (22 isolates, 32.8%) were the most common. In contrast, the IRT-producing isolates were most commonly ST131 (8 isolates, 17.8%), ST73 (5 isolates, 11.1%), and ST23 (5 isolates, 11.1%). Finally, ST73 (12 isolates, 21.4%) and ST95 (5 isolates, 8.9%) were the most common among the AMC-susceptible isolates (Table 2). Four novel STs were identified: STs 3292, 3312, and 3361 in the AMC-susceptible group and STs 2817 and 3312 in the IRT-producing group.

To determine whether a specific sequence type was significantly correlated with an AMC resistance mechanism, we conducted further statistical analysis. We found that a number of sequence types were significantly more prevalent in one specific

group. ST131 was more prevalent in the OXA-1-producing group ($P = 0.0001$) and in the IRT-producing group ($P = 0.06$) than in the AMC-susceptible group (Table 1). ST88 was significantly more prevalent in the OXA-1-producing group than in the IRT-producing and AMC-susceptible groups ($P < 0.0001$) (Table 1). ST73 was significantly more prevalent in the AMC-susceptible group than in the OXA-1-producing group ($P = 0.0005$) (Table 1). ST131 and ST73

TABLE 2 Distribution of STs and phylogenetic groups among IRT-producing, OXA-1-producing, and susceptible isolates[a]

Resistance mechanism (no. of isolates)	ST complex (no. of isolates)	ST(s) (no. of isolates, phylogenetic group)
IRT		
TEM-40 (15)	ST12 (1)	12 (1, B2)
	ST38 (1)	38 (1, D)
	ST73 (2)	73 (2, B2)
	Singletons (11)	131 (3, B2), 117 (2, F), 372 (2, B2), 491 (2, B2), 1193 (1, B2), 3312 (1, B2)
TEM-30 (13)	ST12 (2)	12 (2, B2)
	ST23 (2)	23 (1, C), 410 (1, C)
	ST38 (1)	38 (1, D)
	ST73 (2)	73 (2, B2)
	ST155 (1)	155 (1, B1)
	Singletons (5)	127 (1, B2), 131 (3, B2), 767 (1, B1)
Other IRT (17)[b]	ST10 (2)	10 (1, C), 167 (1, C)
	ST38 (2)	38 (2, D)
	ST73 (2)	73 (1, B2), 156 (1, B1)
	ST23 (4)	23 (4, C)
	ST95 (1)	95 (1, B2)
	Singletons (6)	131 (2, B2), 500 (1, B1), 949 (1, B1), 1196 (1, B1), 2817 (1, A)
OXA-1	ST23 (30)	88 (25, C), 23 (2, C), 90 (1, C), 410 (2, C)
	ST10 (5)	10 (3, C), 167 (1, C), 617 (1, C)
	ST38 (1)	38 (1, D)
	ST73 (1)	73 (1, B2)
	ST155 (1)	58 (1, B1)
	ST156 (1)	156 (1, B1)
	ST448 (1)	448 (1, B1)
	Singletons (27)	131 (22, B2), 224 (1, A), 648 (1, D), 1412 (1, D), 2815 (1, C), 2816 (1, D)
Susceptible	ST73 (12)	73 (12, B2)
	ST95 (5)	95 (5, B2)
	ST10 (4)	10 (4, C)
	ST12 (2)	12 (2, B2)
	ST14 (1)	14 (1, B2)
	ST38 (1)	38 (1, D)
	ST59 (1)	59 (1, F)
	ST69 (1)	69 (1, F)
	ST101 (1)	101 (1, B1)
	ST168 (1)	93 (1, A)
	Singletons (27)	62 (1, F), 91 (1, B2), 127 (1, B2), 131 (3, B2), 141 (3, B2), 224 (1, B2), 372 (1, B2), 420 (1, F), 681 (1, B2), 747 (1, B2), 971 (1, B1), 978 (1, B2), 1057 (1, B2), 1304 (1, B1), 1571 (1, A), 1829 (1, D), 2013 (1, B2), 2230 (1, C), 2346 (1, B2), 3018 (1, D), 3292 (1, B2), 3312 (1, B2), 3361 (1, B2)

[a] IRT, inhibitor-resistant TEM; ST, sequence type.
[b] Five TEM-33 isolates, four TEM-185 isolates, two each isolates of TEM-32 and TEM-34, and one isolate each of TEM-35, TEM-54, TEM-76, and TEM-79.

isolates were detected in all seven participating hospitals, and ST88 was detected in five of them.

Isolates of ST131, ST73, and ST95 belonged to phylogroup B2, and isolates of ST88, ST23, and ST10 belonged to phylogroup C.

Most OXA-1- and CTX-M-15-producing isolates belonged to ST131 (18/25 isolates, 72%), while isolates producing only OXA-1 and not CTX-M-15 mainly belonged to ST88 (25/48 isolates, 52.1%).

All except four isolates of group B2 and ST131 (B2/ST131) belonged to serotype O25b, three of the non-O25b isolates (two IRT producers and one AMC-susceptible isolate) were serotype O16, and the other one was serotype non-O25b non-O16.

Virulence factors. To determine whether there was a relationship between virulence factors and resistance mechanisms, we thoroughly screened the isolates for 49 different VFs and analyzed their virulence relative to the AMC resistance mechanisms present. Data showing the virulence gene content among OXA-1-producing, IRT-producing, and AMC-susceptible isolates are summarized in Table 3. Most of the 49 VFs studied were more frequently detected in the AMC-susceptible isolates than in the OXA-1- or IRT-producing isolates. Nineteen VFs were significantly associated with the AMC-susceptible group, whereas only six were associated with the OXA-1 group ($P = 0.005$; Table 3). Overall, the AMC-susceptible group exhibited a significantly higher virulence score than the OXA-1 group (mean virulence score, 12.5 versus 8.3; $P < 0.0001$). In relation to the AMC-susceptible group and the group producing IRTs, 12 VFs were significantly associated with the AMC-susceptible group, whereas only 1 was associated with the IRT group ($P < 0.001$; Table 3); the AMC-susceptible group also exhibited a significantly higher virulence score than the IRT group (mean score, 12.5 versus 8.2; $P < 0.0001$). Overall, these data suggest that AMC-susceptible isolates may have a high potential for virulence.

Virulence factors in B2 and non-B2 isolates. In order to determine whether there was a relationship between phylogroups and virulence gene content, we next analyzed these data relative to each phylogroup. All isolates, whether they were AMC susceptible or AMC resistant, that belonged to phylogroup B2 exhibited a higher number of VFs and, consequently, a higher virulence score than non-B2 strains (see Table S1 in the supplemental material). The highest virulence scores were observed in the group B2 AMC-susceptible isolates, which possessed a mean VF score of 13.5, whereas the non-B2 AMC-susceptible isolates had a mean VF score of 10.2 ($P = 0.021$). The lowest virulence scores were observed in the non-B2 AMC-resistant isolates, and within this group, the OXA-1 and IRT producers possessed mean virulence scores of 7.7 and 7.3, respectively. The virulence gene content of the isolates belonging to phylogenetic groups B2 (53.4%) and non-B2 (46.6%) in relation to the AMC resistance mechanism is summarized in Table S1 in the supplemental material. Overall, we found that, as expected, phylogroup B2 contained the largest number of virulence factors and the highest virulence score.

Virulence factors in isolates of the most prevalent sequence types. To determine whether there was a relationship between the most prevalent clones detected and the virulence traits, we next performed a statistical analysis of these parameters, as shown in Table 3. ST131 isolates exhibited virulence scores and a range of VFs (mean, 9.1; range, 5 to 13) similar to those of isolates belonging to ST88 (mean, 9.2; range, 5 to 13). ST73 isolates possessed the highest virulence score (mean, 13.2; range, 8 to 18). There were

more virulence factors statistically associated with B2/non-ST131 isolates than with B2/ST131 isolates (13 versus 6), and the virulence score was significantly higher for the former isolates (means, 12.5 and 9.1, respectively; $P < 0.001$). In contrast, the analysis comparing B2/ST73 and B2/non-ST73 isolates revealed that the former had a significantly higher virulence score than the latter (means, 13.2 and 10.7, respectively; $P = 0.003$) (Table 3). In relation to the ST88 clone, C/ST88 isolates possessed a higher virulence score than C/non-ST88 isolates (means, 9.2 and 6.6, respectively; $P = 0.004$) (Table 3).

Among ST131 isolates, those of serotypes O16 and O25b showed similar virulence scores and ranges of VFs (mean scores, 9.3 [range, 7 to 12] and 9.2 [range 5 to 13], respectively).

DISCUSSION

To our knowledge, this is the first study comparing the population structure and virulence-associated genes of AMC-resistant *E. coli* isolates producing either IRTs or OXA-1 with those of AMC-susceptible isolates. Historically, the population of *E. coli*, both environmental and human associated, has been genetically very diverse (23); however, the emergence and dissemination of multiresistant and virulent clones of ExPEC have recently been described (24, 25) and have mainly been associated with the successful B2/ST131 clone (25, 26). Our data suggest that AMC-resistant *E. coli* isolates have a different and less diverse population structure than AMC-susceptible *E. coli* isolates, mainly due to the OXA-1-producing isolates. The mechanism by which antibiotic consumption leads to the selection of certain clones is poorly understood; a recent study suggests that different clones of *E. coli* vary markedly in their response to antibiotics, despite comparable MICs; these results seem to support the ability of antibiotics to select certain successful clones (27).

The association between antimicrobial resistance and virulence in *E. coli* is a controversial topic (9–11, 25, 26). We observed an inverse relationship between resistance to AMC due to OXA-1 and IRT production and virulence potential. These results are in agreement with those of previous studies concluding that *E. coli* resistance to nonfluorinated quinolones, fluoroquinolones, or trimethoprim-sulfamethoxazole is associated with reductions in the virulence traits of the isolates (9–11). In contrast, *E. coli* multiresistant and virulent clones have been described in past years (24–26).

The pandemic clone B2/ST131, previously associated with multiple mechanisms of antibiotic resistance (25), was predominant in the isolates producing both OXA-1 and IRTs, but it was uncommon in AMC-susceptible isolates. The virulence profile of B2/ST131 isolates observed in this study was similar to that of other B2/ST131 isolates in Spain producing other resistance mechanisms, such as an extended-spectrum β-lactamase (12, 13). However, the virulence score of B2/ST131 isolates was lower than that of other B2 isolates not belonging to ST131 (means, 9.10 and 12.5, respectively), which were mainly found in the AMC-susceptible group. This finding is in agreement with that of a previous study suggesting that ST131 isolates could be less virulent than was previously supposed and less virulent than other B2/non-ST131 clones (14).

Although the genetic diversity detected in AMC-susceptible isolates was great, the high prevalence of isolates belonging to the B2/ST73 clone in this AMC-susceptible group (21.4% of all susceptible isolates) is remarkable. The ST73 lineage has recently

TABLE 3 Distribution of virulence determinants among IRT-producing, OXA-1-producing, and AMC-susceptible isolates

Virulence determinant[a]	No. (%) of the following isolates with virulence determinant:									P value					
	Susceptible (n = 56)	OXA-1 (n = 67)	IRT (n = 45)	B2/ST131 (n = 33)	B2/ST73 (n = 18)	C/ST88 (n = 25)	B2/non-ST131 (n = 53)	B2/non-ST73 (n = 68)	C/non-ST88 (n = 23)	Susceptible vs OXA-1	Susceptible vs IRT	B2/ST131 vs B2/non-ST131	B2/ST73 vs B2/non-ST73	B2/ST131 vs B2/ST73	C/ST88 vs C/non-ST88
Adhesins															
papA	20 (35.7)	15 (22.4)	12 (26.7)	7 (21.2)	6 (33.3)	10 (40)	22 (41.5)	23 (33.8)	3 (13)						
papC	22 (39.3)	31 (46.3)	16 (35.6)	9 (27.3)	7 (38.9)	20 (80)	24 (45.3)	26 (38.2)	2 (8.7)						<0.001
papEF	19 (33.9)	5 (7.5)	7 (15.6)	5 (15.2)	6 (33.3)	1 (4)	21 (39.6)	20 (29.4)	2 (8.7)	0.001	0.041	0.018			
papG	20 (35.7)	11 (16.4)	11 (24.4)	7 (21.2)	8 (44.4)	4 (16)	22 (41.5)	21 (30.9)	1 (4.3)	<0.001					
papG I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)						
papG I'	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)	1 (1.5)	0 (0)						
papG II	5 (8.9)	6 (9)	10 (22.2)	5 (15.2)	4 (22.2)	1 (4)	12 (22.6)	13 (19.1)	0 (0)						
papG III	14 (25)	6 (9)	1 (2.2)	2 (6.1)	4 (22.2)	4 (16)	9 (17)	7 (10.3)	1 (4.3)	0.012	<0.001				
fimH	54 (96.4)	63 (94)	44 (97.8)	33 (100)	18 (100)	25 (100)	53 (100)	68 (100)	18 (78.3)						0.020
afa	0 (0)	11 (16.4)	2 (4.4)	9 (27.3)	0 (0)	1 (4)	0 (0)	9 (13.2)	2 (8.7)	0.002		<0.001		0.019	
afaE8	0 (0)	22 (32.8)	1 (2.2)	2 (6.1)	0 (0)	19 (76)	0 (0)	2 (2.9)	0 (0)	<0.001					<0.001
sfa	27 (48.2)	1 (1.5)	4 (8.9)	1 (3)	6 (33.3)	0 (0)	20 (37.7)	15 (22.1)	4 (17.4)	<0.001	<0.001	<0.001		0.035	0.046
focG	10 (17.9)	15 (22.4)	4 (28.9)	8 (24.2)	10 (55.6)	4 (16)	13 (24.5)	11 (16.2)	7 (30.4)				0.001		
f17	0 (0)	0 (0)	0 (0)	1 (3)	1 (5.6)	0 (0)	3 (5.7)	3 (4.4)	0 (0)						
clpG	5 (8.9)	0 (0)	0 (0)	16 (48.5)	3 (16.7)	2 (8)	9 (17)	22 (32.4)	0 (0)	0.013		0.003			
iha	5 (8.9)	16 (23.9)	11 (24.4)	5 (15.2)	6 (33.3)	16 (64)	19 (35.8)	18 (26.5)	2 (8.7)			0.049		0.035	<0.001
hra	27 (48.2)	26 (38.8)	10 (22.2)	2 (6.1)	4 (22.2)	11 (44)	16 (30.2)	14 (20.6)	7 (30.4)		0.007	0.0071			
bmaE	25 (44.6)	16 (23.9)	2 (6.7)	0 (0)	1 (5.6)	1 (4)	1 (1.9)	0 (0)	7 (30.4)	0.020	<0.001				0.020
gafD	0 (0)	2 (3)	1 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)						
Siderophores															
fyuA	27 (48.2)	27 (40.3)	12 (26.7)	19 (57.6)	6 (33.3)	7 (28)	20 (37.7)	33 (48.5)	11 (47.8)		0.039	0.049	0.032	0.041	
iroN	28 (50)	21 (31.3)	10 (22.2)	5 (15.2)	8 (44.4)	12 (48)	23 (43.4)	20 (29.4)	7 (30.4)	0.042	0.006	0.009			
ireA	9 (16.1)	1 (1.5)	3 (6.7)	0 (0)	5 (27.8)	0 (0)	11 (20.8)	6 (8.8)	0 (0)	0.002		0.006	0.001	0.004	
iutA	19 (33.9)	43 (64.2)	20 (44.4)	19 (57.6)	6 (33.3)	18 (72)	16 (30.2)	29 (42.6)	8 (34.8)	0.001		0.014	0.047		0.019
Toxins															
hlyD	16 (28.6)	6 (9)	11 (24.4)	6 (18.2)	8 (44.4)	1 (4)	20 (37.7)	18 (26.5)	1 (4.3)	0.003					
hlyF	5 (8.9)	22 (32.8)	14 (31.1)	7 (21.2)	0 (0)	12 (48)	5 (9.4)	12 (17.6)	10 (43.5)	0.003	0.010		0.001	0.043	
cnf1	4 (7.1)	3 (4.5)	5 (11.1)	2 (6.1)	4 (22.2)	2 (8)	5 (9.4)	3 (4.4)	1 (4.3)						
cdtB	4 (7.1)	1 (1.5)	3 (6.7)	0 (0)	6 (33.3)	0 (0)	8 (15.1)	2 (2.9)	0 (0)			0.021	0.001	0.001	
clbB	17 (30.4)	1 (1.5)	8 (17.8)	1 (3)	8 (44.4)	0 (0)	20 (37.7)	13 (19.1)	2 (8.7)	<0.001		<0.001	0.035	<0.001	
clbN	31 (55.4)	2 (3)	12 (26.7)	2 (6.1)	17 (94.4)	0 (0)	39 (73.6)	24 (35.3)	1 (4.3)	<0.001		<0.001	0.026	<0.001	
astA	6 (10.7)	1 (1.5)	7 (15.6)	0 (0)	2 (11.1)	0 (0)	6 (11.3)	4 (5.9)	4 (17.4)	0.042	0.004				
vat	11 (19.6)	11 (16.4)	11 (24.4)	7 (21.2)	6 (33.3)	3 (12)	16 (30.2)	17 (25)	1 (4.3)						0.046
tsh	4 (7.1)	0 (0)	3 (6.7)	0 (0)	0 (0)	0 (0)	3 (5.7)	3 (4.4)	3 (13)	0.032					
sat	10 (17.9)	22 (32.8)	11 (24.4)	23 (69.7)	8 (44.4)	3 (12)	12 (22.6)	27 (39.7)	1 (4.3)	<0.001	<0.001	<0.001	0.005	0.001	
pic	14 (25)	0 (0)	0 (0)	0 (0)	6 (33.3)	0 (0)	10 (18.9)	4 (5.9)	2 (8.7)	<0.001		0.012			
Capsules															
kpsM II	36 (64.3)	19 (28.4)	21 (46.7)	15 (45.5)	15 (83.3)	4 (16)	41 (77.4)	36 (52.9)	5 (21.7)	<0.001		0.005	0.029	0.016	
kpsM II K1	14 (25)	1 (1.5)	6 (13.3)	2 (6.1)	0 (0)	1 (4)	13 (24.5)	15 (22.1)	1 (4.3)	<0.001		0.040	0.033		
kpsM II K2	8 (14.3)	14 (20.9)	3 (6.7)	9 (27.3)	4 (22.2)	3 (12)	9 (17)	14 (20.6)	3 (13)						
kpsM II K5	2 (3.6)	1 (1.5)	4 (8.9)	0 (0)	2 (11.1)	1 (4)	4 (7.5)	2 (2.9)	1 (4.3)						
kpsM III	1 (1.8)	1 (1.5)	1 (2.2)	1 (3)	0 (0)	0 (0)	1 (1.9)	2 (2.9)	0 (0)						
kpsM K15	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.9)	1 (1.5)	0 (0)						
Miscellaneous															
iss	8 (14.3)	30 (44.8)	11 (24.4)	4 (12.1)	1 (5.6)	18 (72)	9 (17)	12 (17.6)	8 (34.8)	0.001					0.019
cvaC	1 (1.8)	15 (22.4)	1 (2.2)	2 (6.1)	0 (0)	10 (40)	1 (1.9)	3 (4.4)	2 (8.7)	<0.001					0.019
traT	16 (28.6)	33 (49.3)	18 (40)	16 (48.5)	6 (33.3)	14 (56)	21 (39.6)	31 (45.6)	3 (13)	0.026					0.003

Gene												
rfc	4 (7.1)	1 (1.5)	0 (0)	0 (0)	1 (5.6)	1 (4)	4 (7.5)	3 (4.4)	0 (0)	<0.001	<0.001	0.008
ompT	31 (55.4)	9 (13.4)	9 (27.3)	9 (20)	15 (83.3)	2 (8)	26 (49.1)	20 (29.4)	2 (8.7)	<0.001	<0.001	0.002
fliC$_{H7}$	6 (10.7)	0 (0)	0 (0)	0 (0)	1 (5.6)	0 (0)	5 (9.4)	4 (5.9)	0 (0)	<0.001		
ibeA	45 (80.4)	2 (3)	4 (12.1)	5 (11.1)	10 (55.6)	0 (0)	32 (60.4)	26 (38.2)	6 (26.1)	<0.001	<0.001	<0.001
malX	38 (67.9)	30 (44.8)	29 (87.9)	26 (57.89)	13 (72.2)	4 (16)	41 (77.4)	57 (83.8)	6 (26.1)	<0.001	<0.001	<0.001
usp	38 (67.9)	17 (25.4)	21 (63.6)	13 (28.9)	14 (77.8)	3 (12)	37 (69.8)	44 (64.7)	1 (4.3)	<0.001	<0.001	<0.001
Mean (range) virulence score[b]	12.5 (1–19)	8.3 (1–13)	9.1 (5–13)	8.2 (2–17)	13.2 (8–18)	9.2 (5–13)	12.5 (4–19)	10.7 (4–19)	6.6 (1–15)	<0.001	0.003	0.004

[a] Adhesins *fimH* (mannose-specific adhesin of type 1 fimbriae), *papA*, *papC*, *papEF*, and *papG* alleles I, I', II, and III (P fimbria subunits), *sfaS* (S fimbrial adhesin), *focG* (putative F1C fimbrial adhesin), *afa* (Dr antigen-specific adhesin), *afaE8* (afimbrial adhesin VIII), *iha* (nonhemagglutinin adhesin), *bmaE* (blood group M-specific adhesin), *gafD* (glucosamine-specific G fimbriae), *f17* (F17c fimbriae), *clpG* (CS31A adhesin), and *hra* (heat-resistant agglutinin); toxins *hlyD* (α-hemolysin), *hlyF* (hemolysin F), *cnf1* (cytotoxic necrotizing factor 1), *cdtB* (cytolethal distending toxin), *astA* (enteroaggregative *E. coli* heat-stable toxin), *vat* (serine protease), *tsh* (temperature-sensitive hemagglutinin–serine protease), *sat* (secreted autotransporter toxin), and *pic* (serine protease); siderophores *fyuA* (yersiniabactin), *iutA* (aerobactin), and *iroN* and *ireA*; capsule synthesis–associated genes *kpsM* (groups II and III) specifically targeting the K1, K2, and K5 genes of group II capsules, as well as K15; and the miscellaneous VF genes *iss* (surface exclusion serum survival protein), *cvaC* (colicin V from serum resistance-associated plasmids), *traT* (serum resistance), *rfc* (O4 lipopolysaccharide synthesis), *ompT* (protease), *fliC*$_{H7}$ (H7 flagellin variant), *ibeA* (invasion of brain endothelium), *usp* (uropathogenic-specific protein), and *malX* (pathogenicity island marker from the archetypal ExPEC strain CFT073).

[b] The virulence score was the number of virulence genes detected, adjusted for multiple detection of the *pap*, *sfa* and *foc*, *clbB* and *clbN*, and *kpsM* II operons. Virulence scores were compared by use of the Mann-Whitney U test.

been found to be one of the most prevalent STs in uropathogenic isolates in England (16.6% of 300 isolates) (28) and in isolates causing spontaneous bacterial peritonitis and bacteremia in patients with cirrhosis in France (8% of 110 isolates) (29). Most ST73 isolates were antibiotic susceptible, in accordance with the findings of previous studies (28, 29); however, ST73 has also been associated with the production of ESBLs of the CTX-M type in Egypt and Japan (30, 31). In addition, our study showed that isolates belonging to ST73 exhibited the highest virulence score (mean, 13.2; Table 3), in agreement with the findings of another study showing that ST73 was one of the most virulent clones detected in the United Kingdom (28). Although several authors have demonstrated that the overall virulence score of an *E. coli* isolate is directly related to its ability to cause invasive infections and lethality (32, 33), a single, specific VF may enhance the virulence potential of a defined strain (34, 35) beyond the virulence score.

As we described previously (5), 37.3% of the OXA-1-producing isolates belonged to ST88. ST88 has also been described in association with chromosome-mediated AmpC overproduction in a French hospital (36), but so far its virulence profile had not been reported. Our ST88 isolates belonged to the recently proposed phylogroup C, and they possessed a high virulence score (mean, 9.2). Most of the virulence-associated traits of isolates belonging to ST88 were adhesins, protectins, and siderophores that may facilitate persistence and survival in adverse circumstances. Phylogroup C has previously been identified in a virulent strain causing an outbreak in a neonatal ward (37). Interestingly, in this study, 30% of *E. coli* isolates belonged to phylogroup C, mainly due to the reclassification of prevalent clonal complexes ST10 and ST23 (Table 2) previously classified as phylogroup A by the former method of Clermont et al. (19). In this study, carried out in clinical isolates, phylogroup A was very uncommon (2.4%), in contrast to the 18 to 28% prevalence described previously in two different collections of human fecal isolates (15).

Concluding remarks. Our findings suggest that IRT- and OXA-1-producing *E. coli* isolates resistant to AMC have a population structure different from and less diverse than that of AMC-susceptible clinical *E. coli* isolates, mainly due to OXA-1 producers. AMC-susceptible isolates had more VFs than AMC-resistant isolates. We also provide information about the higher numbers of virulence traits in the B2/ST73 clone than the B2/ST131 and C/ST88 clones.

REFERENCES

1. **Oteo J, Lázaro E, de Abajo FJ, Baquero F, Campos J, and Spanish Members of EARSS.** 2005. Antimicrobial-resistant invasive *Escherichia coli*, Spain. Emerg. Infect. Dis. **1**:546–553. http://dx.doi.org/10.3201/eid1104.040699.

2. **Campos J, Ferech M, Lázaro E, de Abajo F, Oteo J, Stephens P, Goossens H.** 2007. Surveillance of outpatient antibiotic consumption in Spain according to sales data and reimbursement data. J. Antimicrob. Chemother. **60**:698–701. http://dx.doi.org/10.1093/jac/dkm248.

3. **Mimica Matanovic S, Bergman U, Vukovic D, Wettermark B, Vlahovic-Palcevski V.** 2010. Impact of restricted amoxicillin/clavulanic acid use on *Escherichia coli* resistance-antibiotic DU90% profiles with bacterial resistance rates: a visual presentation. Int. J. Antimicrob. Agents **36**:369–373. http://dx.doi.org/10.1016/j.ijantimicag.2010.05.019.

4. **Oteo J, Campos J, Lázaro E, Cuevas O, García-Cobos S, Pérez-Vázquez M, de bajo FJ, Spanish Members of EARSS.** 2008. Increased amoxicillin-clavulanic acid resistance in *Escherichia coli* blood isolates, Spain. Emerg. Infect. Dis. **14**:1259–1262. http://dx.doi.org/10.3201/eid1408.071059.

5. **Ortega A, Oteo J, Aranzamendi-Zaldumbide M, Bartolomé RM, Bou G, Cercenado E, Conejo MC, González-López JJ, Marín M, Martínez-Martínez L, Merino M, Navarro F, Oliver A, Pascual A, Rivera A, Rodríguez-Baño J, Weber I, Aracil B, Campos J.** 2012. Spanish multicenter study of the epidemiology and mechanisms of amoxicillin-clavulanate resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **12**:576–581. http://dx.doi.org/10.1128/AAC.06393-11.

6. **Rodríguez-Baño J, Oteo J, Ortega A, Villar M, Conejo MC, Bou G, Aranzamendi-Zaldumbide M, Cercenado E, Gurguí M, Martínez-Martínez L, Merino M, Rivera A, Oliver A, Weber I, Pascual A, Bartolomé RM, Gónzalez-López JJ, Campos J.** 2013. Epidemiological and clinical complexity of amoxicillin-clavulanate-resistant *Escherichia coli*. J. Clin. Microbiol. **51**:2414–2417. http://dx.doi.org/10.1128/JCM.00999-13.

7. **Moreno E, Prats G, Planells I, Planes AM, Perez T, Andreu A.** 2006. Characterization of *Escherichia coli* isolates derived from phylogenetic groups A and B1 causing extraintestinal infection. Enferm. Infecc. Microbiol. Clin. **24**:483–489. (In Spanish.) http://dx.doi.org/10.1157/13092463.

8. **Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventre A, Elion J, Picard D, Denamur E.** 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology **147**:1671–1676.

9. **Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A.** 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. J. Antimicrob. Chemother. **57**:204–211. http://dx.doi.org/10.1093/jac/dki468.

10. **Horcajada JP, Soto S, Gajewski A, Smithson A, Jiménez de Anta MT, Mensa J, Vila J, Johnson JR.** 2005. Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J. Clin. Microbiol. **43**:2962–2964. http://dx.doi.org/10.1128/JCM.43.6.2962-2964.2005.

11. **Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, Moreno A, Mensa J.** 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J. Infect. Dis. **186**:1039–1042. http://dx.doi.org/10.1086/342955.

12. **Blanco J, Mora A, Mamani R, López C, Blanco M, Dahbi G, Herrera A, Blanco JE, Alonso MP, García-Garrote F, Chaves F, Orellana MÁ, Martínez-Martínez L, Calvo J, Prats G, Larrosa MN, González-López JJ, López-Cerero L, Rodríguez-Baño J, Pascual A.** 2011. 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. J. Antimicrob. Chemother. **66**:2011–2121. http://dx.doi.org/10.1093/jac/dkr235.

13. **Coelho A, Mora A, Mamani R, López C, González-López JJ, Larrosa MN, Quintero-Zarate JN, Dahbi G, Herrera A, Blanco JE, Blanco M, Alonso MP, Prats G, Blanco J.** 2011. Spread of *Escherichia coli* O25b:H4-B2-ST131 producing CTX-M-15 and SHV-12 with high virulence gene content in Barcelona (Spain). J. Antimicrob. Chemother. **66**:517–526. http://dx.doi.org/10.1093/jac/dkq491.

14. **Lavigne JP, Vergunst AC, Goret L, Sotto A, Combescure C, Blanco J, O'Callaghan D, Nicolas-Chanoine MH.** 2012. Virulence potential and genomic mapping of the worldwide clone *Escherichia coli* ST131. PLoS One **7**:e34294. http://dx.doi.org/10.1371/journal.pone.0034294.

15. **Clermont O, Christenson JK, Denamur E, Gordon DM.** 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. **5**:58–65. http://dx.doi.org/10.1111/1758-2229.12019.

16. **Woodford N, Turton JF, Livermore DM.** 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. **35**:736–755. http://dx.doi.org/10.1111/j.1574-6976.2011.00268.x.

17. **Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G.** 2008. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J. Antimicrob. Chemother. **61**:1024–1028. http://dx.doi.org/10.1093/jac/dkn084.

18. **Clermont O, Johnson JR, Menard M, Denamur E.** 2007. Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. Diagn. Microbiol. Infect. Dis. **57**:129–136. http://dx.doi.org/10.1016/j.diagmicrobio.2006.08.007.

19. **Clermont O, Bonacorsi S, Bingen E.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66**:4555–4558. http://dx.doi.org/10.1128/AEM.66.10.4555-4558.2000.

20. **Sannes MR, Kuskowski MA, Owens K, Gajewski A, Johnson JR.** 2004. Virulence factor profiles and phylogenetic background of *Escherichia coli* isolates from veterans with bacteremia and uninfected control subjects. J. Infect. Dis. **190**:2121–2128. http://dx.doi.org/10.1086/425984.

21. **Johnson JR, Stell AL.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181**:261–272. http://dx.doi.org/10.1086/315217.

22. **Johnson JR, Johnston B, Kuskowski MA, Nougayrede JP, Oswald E.** 2008. Molecular epidemiology and phylogenetic distribution of the *Escherichia coli* pks genomic island. J. Clin. Microbiol. **46**:3906–3911. http://dx.doi.org/10.1128/JCM.00949-08.

23. **González-González A, Sánchez-Reyes LL, Delgado Sapien G, Eguiarte LE, Souza V.** 2013. Hierarchical clustering of genetic diversity associated to different levels of mutation and recombination in *Escherichia coli*: a study based on Mexican isolates. Infect. Genet. Evol. **13**:187–197. http://dx.doi.org/10.1016/j.meegid.2012.09.003.

24. **Mihaila L, Wyplosz B, Clermont O, Garry L, Hipeaux MC, Vittecoq D, Dussaix E, Denamur E, Branger C.** 2010. Probable intrafamily transmission of a highly virulent CTX-M-3-producing *Escherichia coli* belonging to the emerging phylogenetic subgroup D2 O102-ST405 clone. J. Antimicrob. Chemother. **65**:1537–1539. http://dx.doi.org/10.1093/jac/dkq155.

25. **Oteo J, Pérez-Vázquez M, Campos J.** 2010. Extended-spectrum β-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. Curr. Opin. Infect. Dis. **23**:320–326. http://dx.doi.org/10.1097/QCO.0b013e3283398dc1.

26. **Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G.** 2008. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J. Antimicrob. Chemother. **61**:1024–1028. http://dx.doi.org/10.1093/jac/dkn084.

27. **Stewart B, Rozen DE.** 2012. Genetic variation for antibiotic persistence in *Escherichia coli*. Evolution **66**:933–939. http://dx.doi.org/10.1111/j.1558-5646.2011.01467.x.

28. **Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M.** 2012. Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from northwest England. J. Antimicrob. Chemother. **67**:346–356. http://dx.doi.org/10.1093/jac/dkr451.

29. **Bert F, Johnson JR, Ouattara B, Leflon-Guibout V, Johnston B, Marcon E, Valla D, Moreau R, Nicolas-Chanoine MH.** 2010. Genetic diversity and virulence profiles of *Escherichia coli* isolates causing spontaneous bacterial peritonitis and bacteremia in patients with cirrhosis. J. Clin. Microbiol. **48**:2709–2714. http://dx.doi.org/10.1128/JCM.00516-10.

30. **Fam N, Leflon-Guibout V, Fouad S, Aboul-Fadl L, Marcon E, Desouky D, El-Defrawy I, Abou-Aitta A, Klena J, Nicolas-Chanoine MH.** 2011. CTX-M-15-producing *Escherichia coli* clinical isolates in Cairo (Egypt), including isolates of clonal complex ST10 and clones ST131, ST73, and ST405 in both community and hospital settings. Microb. Drug Resist. **17**:67–73. http://dx.doi.org/10.1089/mdr.2010.0063.

31. **Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y.** 2009. Change in the prevalence of extended-spectrum-beta-lactamase-

producing *Escherichia coli* in Japan by clonal spread. J. Antimicrob. Chemother. **63:**72–79. http://dx.doi.org/10.1093/jac/dkn463.

32. **Kudinha T, Johnson JR, Andrew SD, Kong F, Anderson P, Gilbert GL.** 2013. Distribution of phylogenetic groups, sequence type ST131, and virulence-associated traits among *Escherichia coli* isolates from men with pyelonephritis or cystitis and healthy controls. Clin. Microbiol. Infect. **19:**E173–E180. http://dx.doi.org/10.1111/1469-0691.12123.

33. **Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur E.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.

34. **Johnson JR, Porter SB, Zhanel G, Kuskowski MA, Denamur E.** 2012. Virulence of *Escherichia coli* clinical isolates in a murine sepsis model in relation to sequence type ST131 status, fluoroquinolone resistance, and virulence genotype. Infect. Immun. **80:**1554–1562. http://dx.doi.org/10.1128/IAI.06388-11.

35. **Johnson JR, Clermont O, Menard M, Kuskowski MA, Picard B, Denamur E.** 2006. Experimental mouse lethality of *Escherichia coli* isolates, in relation to accessory traits, phylogenetic group, and ecological source. J. Infect. Dis. **194:**1141–1150. http://dx.doi.org/10.1086/507305.

36. **Crémet L, Caroff N, Giraudeau C, Dauvergne S, Lepelletier D, Reynaud A, Corvec S.** 2010. Occurrence of ST23 complex phylogroup A *Escherichia coli* isolates producing extended-spectrum AmpC beta-lactamase in a French hospital. Antimicrob. Agents Chemother. **54:**2216–2218. http://dx.doi.org/10.1128/AAC.01580-09.

37. **Moissenet D, Salauze B, Clermont O, Bingen E, Arlet G, Denamur E, Mérens A, Mitanchez D, Vu-Thien H.** 2010. Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum β-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. J. Clin. Microbiol. **48:**2459–2463. http://dx.doi.org/10.1128/JCM.00529-10.