Applied and Environmental Microbiology, Nov. 2010, p. 6991–6997 Vol. 76, No. 21
0099-2240/10/$12.00 doi:10.1128/AEM.01112-10

Recent Emergence of Clonal Group O25b:K1:H4-B2-ST131 *ibeA* Strains among *Escherichia coli* Poultry Isolates, Including CTX-M-9-Producing Strains, and Comparison with Clinical Human Isolates[▽]

Azucena Mora,[1] Alexandra Herrera,[1] Rosalia Mamani,[1] Cecilia López,[1] María Pilar Alonso,[2] Jesús E. Blanco,[1] Miguel Blanco,[1] Ghizlane Dahbi,[1] Fernando García-Garrote,[2] Julia María Pita,[2] Amparo Coira,[2] María Isabel Bernárdez,[1] and Jorge Blanco[1]*

Laboratorio de Referencia de E. coli (LREC), Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain,[1] and Unidade de Microbioloxía Clínica, Complexo Hospitalario Xeral-Calde, Lugo, Spain[2]

Received 7 May 2010/Accepted 24 August 2010

To discern the possible spread of the *Escherichia coli* O25b:H4-ST131 clonal group in poultry and the zoonotic potential of avian strains, we made a retrospective search of our strain collection and compared the findings for those strains with the findings for current strains. Thus, we have characterized a collection of 19 avian O25b:H4-ST131 *E. coli* strains isolated from 1995 to 2010 which, interestingly, harbored the *ibeA* gene. Using this virulence gene as a criterion for selection, we compared those 19 avian strains with 33 human O25b:H4-ST131 *ibeA*-positive *E. coli* strains obtained from patients with extraintestinal infections (1993 to 2009). All 52 O25b:H4-ST131 *ibeA*-positive *E. coli* strains shared the *fimH*, *kpsMII*, *malX*, and *usp* genes but showed statistically significant differences in nine virulence factors, namely, *papGIII*, *cdtB*, *sat*, and *kpsMII* K5, which were associated with human strains, and *iroN*, *kpsMII* K1, *cvaC*, *iss*, and *tsh*, which were associated with strains of avian origin. The XbaI macrorestriction profiles of the 52 *E. coli* O25b:H4-ST131 *ibeA*-positive strains revealed 11 clusters (clusters I to XI) of >85% similarity, with four clusters including strains of human and avian origin. Cluster VII (90.9% similarity) grouped 10 strains (7 avian and 3 human strains) that mostly produced CTX-M-9 and that also shared the same virulence profile. Finally, we compared the macrorestriction profiles of the 12 CTX-M-9-producing O25b:H4-ST131 *ibeA* strains (7 avian and 5 human strains) identified among the 52 strains with those of 15 human O25b:H4-ST131 CTX-M-14-, CTX-M-15-, and CTX-M-32-producing strains that proved to be negative for *ibeA* and showed that they clearly differed in the level of similarity from the CTX-M-9-producing strains. In conclusion, *E. coli* clonal group O25b:H4-ST131 *ibeA* has recently emerged among avian isolates with the new acquisition of the K1 capsule antigen and includes CTX-M-9-producing strains. This clonal group represents a real zoonotic risk that has crossed the barrier between human and avian hosts.

Strains of the extensively antimicrobial-resistant *Escherichia coli* clonal group of sequence type (ST) 131 (ST131) belonging to serotype O25b:H4 have recently been recognized to be important human pathogens worldwide (9, 33). Although it is commonly associated with the dissemination of CTX-M-15 extended-spectrum cephalosporin resistance, *E. coli* O25b:H4-ST131 also occurs as a fluoroquinolone (FQ)-resistant but cephalosporin-susceptible pathogen (5, 22, 26, 27). Currently, it is assumed that O25b:H4-ST131 strains circulate not only among humans but also among animal hosts (13, 21, 37), which would contribute to the ongoing global emergence of O25b:H4-ST131, in the case of regular transmission between animals and humans. Even though CTX-M-15 is the most widely distributed extended-spectrum beta-lactamase (ESBL) linked to this clonal group, other, different variants of CTX-M have recently been reported, such as CTX-M-9, CTX-M-14, and CTX-M-32 (4, 34, 36, 39). Noteworthy was the detection, for the first time on poultry farms, of this clonal group producing CTX-M-9 that had macrorestriction profiles and virulence genes very similar to those observed in clinical human isolates (10).

Extraintestinal pathogenic *E. coli* (ExPEC) strains, which include avian pathogenic *E. coli* (APEC) and human uropathogenic *E. coli* (UPEC), septicemic *E. coli*, and newborn meningitis-causing *E. coli* (NMEC) strains, exhibit considerable genome diversity and have a wide range of virulence-associated factors (12, 18). While infections caused by APEC strains initially start as a respiratory tract disease which evolves to a systemic infection of the internal organs and, finally, to sepsis, the most frequent origin of human sepsis is urinary tract infection (UTI), especially pyelonephritis (2, 3, 11). However, APEC strains have been recognized to share common traits with human isolates (29, 30, 31), including the K1 capsule antigen (23, 24, 29) and the *ibeA* gene (14). In addition, retail chicken products have been found to carry nalidixic-resistant ExPEC strains (17, 19), and although it is drug susceptible, an *E. coli* strain belonging to the O25b:H4-ST131 clonal group has even recently been detected in retail chicken (41), supporting the urgent necessity for the implementation of food control measures.

* Corresponding author. Mailing address: Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, LREC, Universidade de Santiago de Compostela (USC), 27002 Lugo, Spain. Phone and fax: 34 982 285936. E-mail: jorge.blanco@usc.es.

▽ Published ahead of print on 3 September 2010.

TABLE 1. Antibiotic resistance of the 19 avian O25b:H4-ST131 *ibeA*-positive *E. coli* strains

Strain code	Yr	Origin	ESBL type	Antibiotic resistance[a]
FV 9211	1995	Avian pathology associated[b]		CEF, AMP, PIP, TIC, SXT
FV 9212	1995	Avian pathology associated[b]		AMP, PIP, TIC, NAL, SXT
FV 9213	1992	Avian pathology associated[c]		CEF, CFZ, AMP, PIP, TIC, NAL, SXT
FV 9807	2007	Avian pathology associated[c]		
FV 10608	2003	Poultry feces[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC, SXT
FV 11686	2008	Avian pathology associated[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC
FV 11687	2008	Avian pathology associated[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC, TOB
FV 12593	2009	Avian pathology associated[c]		
FV 13455	2009	Avian pathology associated[c]		
FV 14067	2009	Avian pathology associated[c]		
FV 14087	2009	Avian pathology associated[c]		CEF
FV 14287	2009	Avian meat[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC, NAL, SXT
FV 14288	2009	Avian meat[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC, NAL, SXT
FV 14289	2009	Avian meat[c]		CEF, NAL, SXT
FV 14290	2009	Avian meat[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC, NAL, GEN, SXT
FV 14292	2009	Avian meat[c]		CEF, NAL, AMP, PIP, TIC, GEN, TOB, SXT
FV 14293	2009	Avian meat[c]	CTX-M-9	CEF, CFZ, CXM, CTX, FEP, AMP, PIP, TIC
FV 14294	2010	Avian meat[c]		CEF, CFZ, CXM, FOX, AMC, AMP, TIC
FV 14295	2010	Avian meat[c]		CEF, NAL, AMP, PIP, TIC, GEN, TOB, SXT

[a] The antibiotics to which resistance was tested were cephalothin (CEF), cefazolin (CFZ), cefuroxime (CXM), cefotaxime (CTX), cefepime (FEP), cefoxitin (FOX), amoxicillin-clavulanate (AMC), ampicillin (AMP), piperacillin (PIP), ticarcillin (TIC), gentamicin (GEN), tobramycin (TOB), nalidixic acid (NAL), ciprofloxacin (CIP), and trimethoprim-sulfamethoxazole (SXT).
[b] Turkey.
[c] Chicken.

The aim of the present study was to discern the possible spread of the O25b:H4-ST131 clonal group, especially CTX-M-9-producing strains, in poultry and the zoonotic potential of avian isolates. For this purpose, we made a retrospective search of our human and avian strain collections and compared the findings for those strains with the findings for current strains. Identification of this emerging clone among avian sources and comparison of the clone with clinical human isolates will shed new light on the epidemiology of the O25b:H4-ST131 clonal group.

MATERIALS AND METHODS

***E. coli* strains.** In the present study, we have characterized a total of 67 avian and human *E. coli* strains (19 avian and 48 human) belonging to clonal group O25b:H4-B2-ST131.

The 19 poultry O25b:H4-ST131 strains were distributed as follows: 8 strains were isolated from 7 retail chicken samples from among 100 samples obtained from September 2009 to February 2010 in the city of Lugo (northwest Spain) (prevalence, 7%); 10 strains were isolated from birds with different pathologies, with 7 of those 10 strains being obtained from among 463 chicken *E. coli* strains obtained from 2007 to 2009 in Spain (prevalence, 1.5%) and the remaining 3 (2 from turkeys and 1 from a chicken) being obtained from among a collection of 1,601 avian (974 chicken, 408 turkey, and 159 duck) *E. coli* strains isolated from 1991 to 2001 in Spain, France, and Belgium (prevalence, 0.2%) (38); and finally, 1 strain was isolated from among 57 chicken *E. coli* strains obtained from poultry feces in 2003 in Catalonia (northeast Spain) (prevalence, 1.8%) (10).

Forty-eight human O25b:H4-ST131 *E. coli* strains were selected for comparison with those of avian origin: (i) a group of 28 non-ESBL-producing *ibeA*-positive O25b:H4-ST131 strains (prevalence, 1.1%) obtained from among 83 strains belonging to the clonal group (prevalence, 3.4%) recovered from 2,464 *E. coli*-containing cultures of blood from patients admitted to Xeral-Calde Hospital in Lugo from 1993 to 2009 and (ii) an additional group of 20 O25b:H4-ST131 strains producing different CTX-M types obtained from among 761 ESBL-producing *E. coli* strains (143 [18.8%] belonging to the clonal group) from 2006 to 2009 from patients with extraintestinal infections (urinary tract infections or sepsis) admitted to five hospitals in Galicia (northwest Spain).

Identification of O25b:H4-B2-ST131 strains: serotyping, phylogenetic grouping, and MLST. The strains in the source collections were first identified by means of PCR by the detection of the specific O25b molecular subtype (4, 8). Afterwards, the strains were confirmed to be O25:H4 by serotyping, and their molecular characterization was completed. The determination of the O25 and H4 antigens was carried out using the method previously described by Guinée et al. (15) with specific antisera obtained from the Laboratorio de Referencia de *E. coli*, Universidade de Santiago de Compostela. The phylogenetic group of the *E. coli* strains (group B2) was determined by the multiplex PCR-based method of Clermont et al. (7). Multilocus sequence typing (MLST) (ST131) was performed as described previously by gene amplification and sequencing of the seven housekeeping genes (*adk*, *fum*C, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) by use of the protocol and primers specified at the *E. coli* MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli) (28). The allelic profiles (STs) of the seven gene sequences were obtained via the electronic database at the *E. coli* MLST website.

Antibiotic susceptibility testing and ESBL typing. The strains were screened for ESBL production by testing for cephalosporin resistance and performing the double-disk synergy test described by Jarlier et al. (16). MICs were determined by use of the MicroScan WalkAway automated system (Siemens, Spain), according to the manufacturer's instructions. Resistance was interpreted on the basis of the recommended breakpoints of the CLSI (formerly the NCCLS) (32). To determine the genotypes of the ESBLs, PCR was performed using the TEM-, SHV-, CTX-M-1, and CTX-M-9 group-specific primers, as reported previously (25). Sequencing was also performed with the same PCR primers and under the same conditions. The sequences obtained were then compared with those of the corresponding genes available in GenBank.

Virulence genotyping. The strains were analyzed for the presence of virulence genes, as documented elsewhere (4, 29), using primers specific for genes and operons that encode extraintestinal virulence factors characteristic of ExPEC: *fimH*, $fimAv_{MT78}$, *papEF* (strains with positive results for *papEF* were tested for the *papGI*, *papGII*, and *papGIII* alleles), *sfa* and *focDE* (analyses for which were performed together, and strains with positive results were tested for *sfaS* and *focG*), *afa* and *draBC*, *bmaE*, *gafD*, *cnf1*, *cdtB*, *sat*, *hlyA*, *iucD*, *iroN*, *kpsMII* (establishing *neuC* K1, K2, and K5 variants), *kpsMIII*, *cvaC*, *iss*, *traT*, *ibeA*, *malX*, *usp*, and *tsh*.

The extraintestinal pathogenic status of the strains was analyzed according to the definition of Johnson et al. (17). A strain satisfied the criteria for being extraintestinal pathogenic if it carried two or more of the following genes: *pap* (P fimbriae), *sfa* and *focDE* (S/F1C fimbriae), *afa* and *draBC* (Dr binding adhesins), *iucD* (aerobactin receptor), and *kpsMII* (group 2 capsule synthesis).

PFGE. Pulsed-field gel electrophoresis (PFGE) analysis with XbaI digestion was performed as described previously (29). The PFGE profiles were analyzed with the BioNumerics fingerprinting software (Applied Maths, St-Martens-Latem, Belgium). Cluster analysis of the Dice similarity indices based on the unweighted-pair group method using arithmetic linkages (UPGMA) was done to

TABLE 2. Virulence gene characterization of the 52 O25:H4-ST131 *ibeA*-positive *E. coli* strains included in this study[a]

Category	Gene(s)	Comment(s)	No. (%) of strains			*P* value[b]
			Avian strains (n = 19)	Human *ibeA*-positive strains (n = 33)	Avian and human strains (n = 52)	
Adhesins	*fimH*	D-Mannose-specific adhesin, type 1 fimbriae	19 (100)	33 (100)	52 (100)	1.000
	$fimAv_{MT78}$	FimA variant MT78 of type 1 fimbriae	0	0	0	
	pap	Pilus associated with pyelonephritis (P fimbriae)				
	papEF	Pilus assembly, central region of *pap* operon	1 (8.3)	**12 (36.4)**	13 (25)	**0.011**
	papGI	Gal(α1-4) Gal-specific pilus tip adhesin molecule rare	0	0	0	
	papGII	Pyelonephritis-associated gene	1 (8.3)	0	1 (2)	0.365
	papGIII	Cystitis-associated gene	0	**12 (36.4)**	12 (23.1)	**0.002**
	sfa and *focDE*	Central region of *sfa* and *foc* operons	0	0	0	
	afa and *draBC*	Dr antigen-specific adhesin operons (AFA, Dr, F1845)	0	5 (15.1)	5 (9.6)	0.091
	bmaE	Blood group M-specific adhesin	0	1 (3)	1 (1.9)	0.635
	gafD	*N*-Acetyl-D-glucosamine-specific (G, F17c) fimbria adhesin	0	0	0	
Toxins	*cnf1*	Cytotoxic necrotizing factor 1	0	1 (3)	1 (1.9)	0.635
	cdtB	Cytolethal distending toxin	1 (8.3)	**17 (51.5)**	18 (34.6)	**0.001**
	sat	Secreted autotransporter toxin	0	**7 (21.2)**	7 (13.5)	**0.032**
	hlyA	α-Hemolysin	0	1 (3)	1 (1.9)	0.635
Siderophores	*iucD*	Ferric aerobactin receptor (iron uptake, transport)	17 (89.5)	24 (72.7)	41 (78.8)	0.142
	iroN	Novel catecholate siderophore receptor	19 (100)	17 (51.5)	36 (69.2)	**0.000**
Protectins	*kpsMII*	Group II capsule	19 (100)	33 (100)	52 (100)	1.000
	kpsMII K2	K2 group II capsule	0	0	0	
	kpsMII K5	K5 group II capsule	5 (26.3)	**26 (78.8)**	31 (59.6)	**0.000**
	neuC K1	K1 antigen	**14 (73.7)**	7 (21.2)	21 (40.4)	**0.000**
	kpsMII	Group III capsule	0	0	0	
	cvaC	ColV; on plasmids with *traT*, *iss*, and antibiotic resistance genes	**17 (89.5)**	19 (57.6)	36 (69.2)	**0.016**
	iss	Increased serum survival (outer membrane protein)	**19 (100)**	20 (60.6)	39 (75)	**0.001**
	traT	Surface exclusion, serum survival (outer membrane protein)	19 (100)	28 (84.8)	47 (90.4)	0.091
Miscellaneous	*malX* (PAI)	Pathogenicity-associated island marker	19 (100)	33 (100)	52 (100)	1.000
	usp	Uropathogenic strain-specific protein (bacteriocin)	19 (100)	33 (100)	52 (100)	1.000
	tsh	Tsh (temperature-sensitive hemagglutinin) serine protease	**15 (78.9)**	5 (15.1)	20 (38.5)	**0.000**
Mean for virulence genes[c]			10.7	9.7	10.1	

[a] Boldface data indicate statistical significance.
[b] For the avian versus human strains. For each comparison, a *P* value of <0.05 was considered statistically significant, and a *P* value of >0.05 was considered not statistically significant.
[c] Range, 6 to 13.

generate a dendrogram describing the relationship among the PFGE profiles. Isolates were considered related if their Dice similarity index was >85%, according to criteria of Tenover et al. (a difference of six bands or less) (40).

Statistical analysis. For the avian and human O25b:H4-ST131 populations, Fisher's exact test was used to test the null hypothesis that the gene prevalence rates across the two populations studied were equal. For each comparison, a *P* value of <0.05 was considered to denote significant differences.

RESULTS

In the present study, we have detected an increasing presence of the clonal group O25b:H4-ST131 not only in association with avian pathology (0.2% from 1991 to 2001 to 1.5% from 2007 to 2009; $P = 0.002$) but also in retail chicken (7% of samples obtained in 2009 and 2010). The 19 avian O25b:H4-ST131 *E. coli* strains isolated were compared with 48 human strains belonging to the same clonal group.

ESBL production and associated resistance. Twenty-seven of the 67 O25b:H4-ST131 *E. coli* isolates included in the present study were positive for CTX-M-production: 7 of the 19 avian strains were CTX-M-9 producers, and 20 of the 48 human strains produced different CTX-M-types (6 strains produced CTX-M-15, 5 produced CTX-M-14, 5 produced CTX-M-9, 3 produced CTX-M-32, and 1 produced both types CTX-M-14/15).

Table 1 summarizes the antibiotic resistance of the 19 avian strains. Only four strains were susceptible to all antibiotics tested; and high MICs for gentamicin, tobramycin, and ciprofloxacin were detected in all CTX-M-9-producing avian strains.

Virulence genotyping. Interestingly, the 19 avian strains harbored the *ibeA* gene; for this reason, we compared them with 33 human O25b:H4-ST131 *ibeA*-positive *E. coli* strains (28 non-ESBL-producing and 5 CTX-M-9-producing strains) obtained from patients with extraintestinal infections. Table 2 summarizes the virulence genes harbored by the 52 O25b:H4-ST131 *ibeA*-positive *E. coli* strains. Apart from the *ibeA* gene, all 52 strains shared the *fimH*, *kpsMII*, *malX*, and *usp* genes but

showed statistically significant differences in nine virulence factors, namely, *papGIII*, *cdtB*, *sat*, and *kpsMII* K5, which were associated with human strains, and *iroN*, *kpsMII* K1, *cvaC*, *iss*, and *tsh*, which were associated with strains of avian origin.

The number of virulence factors harbored by the 52 O25b:H4-ST131 *ibeA*-positive strains ranged from 6 to 13, and a total of 23 virulence-gene profiles were detected, as shown in Fig. 1. Avian strains showed six different gene profiles, with profile 3 with 11 virulence genes (P3-11) being the most frequently detected (12 strains, 63.2%). Human strains showed 18 different gene profiles, with 1 profile (P3-11, 3 human strains) being shared with avian strains. Forty-six of the 52 O25b:H4-ST131 *ibeA*-positive *E. coli* strains (29 human and 17 avian) satisfied the criteria for extraintestinal pathogenic status.

In contrast to the 12 avian and human CTX-M-9-producing *ibeA*-positive strains, none of the remaining 15 human O25b:H4-ST131 *E. coli* strains producing other CTX-M types (6 producing CTX-M-15, 5 producing CTX-M-14, 3 producing CTX-M-32, and 1 producing CTX-M-14/15) harbored the *ibeA* gene and showed five gene profiles (profiles P24 to P28) not found among the 23 gene profiles of the 52 human and avian O25b:H4-ST131 *ibeA*-positive strains (Fig. 2).

Macrorestriction profiles by PFGE. Figure 1 shows a dendrogram with the XbaI macrorestriction profiles obtained by PFGE of the 52 O25b:H4-ST131 *ibeA*-positive strains analyzed. The strains formed four groups (groups A, B, C, and D) with similarities of 66.8%, 73.5%, 77.7%, and 71%, respectively. Group B included all 21 K1 strains (14 avian and 7 human strains), while the remaining 31 K5 strains (26 human and 5 avian strains) were divided into groups A (mostly *papGIII* positive; 11 of 17 strains), C (mostly *sat* positive; 6 of 7 strains), and D (mostly *cdtB* positive and *papG* negative; 6 of 7 strains). Looking at these four large groups, PFGE revealed 11 clusters (clusters I to XI) of >85% similarity, with 4 clusters (clusters IV, VII, VIII, and XI) including strains of human and avian origin. Cluster VII (90.9% similarity) grouped 10 strains (7 avian and 3 human strains) that mostly produced CTX-M-9 (9 strains) and that also shared the same virulence profile (P3-11).

We wanted to compare the macrorestriction profiles of the 12 CTX-M-9-producing O25b:H4-ST131 strains (7 avian and 5 human strains, all of them *ibeA* positive) characterized in this study with those of 15 human *E. coli* O25b:H4-ST131 strains (all of them *ibeA* negative) producing different types of CTX-M (6 producing CTX-M-15, 5 producing CTX-M-14, 3 producing CTX-M-32, and 1 producing CTX-M-14/15). Figure 2 shows the dendrogram of the 27 CTX-M-producing strains that remained distributed according to the type of CTX-M produced and virulence genes in five groups (groups A, B, C, D, and E) with similarities of 79.8%, 81%, 77.9%, 70.9%, and 90.6%, respectively. Group A included 10 CTX-M-9-producing K1 strains with a cluster of 89.6% similarity, including those 9 CTX-M-9-producing strains with virulence profile P3-11. Group B included the remaining two CTX-M-9-producing strains that harbored the K5 capsule. Group C included seven *afa* and *draBC* K2 strains (six CTX-M-15-producing strains and one CTX-M-32-producing strain) with a cluster of 90.8% similarity, including those six CTX-M-15-producing strains. Group D included five K5 strains with virulence profile P26-7 producing CTX-M-32, CTX-M-14, and CTX-M-14/15 (both types). Finally, group E included three CTX-M-14-producing K5 strains that were *fimH* negative and *afa* and *draBC* positive.

DISCUSSION

Due to the recent report of clonal group O25b:H4-ST131 among *E. coli* strains isolated from poultry feces (10), we made a retrospective search for its presence and also obtained a new sample of strains from clinical avian cases and retail chicken in order to better know the epidemiology of this emerging group. In the present study, we have detected an increasing presence of clonal group O25b:H4-ST131 not only in association with avian pathology but also in association with retail chicken. We also report here the detection of O25b:H4-ST131 *E. coli* strains producing CTX-M-9 from retail chicken as well as from samples associated with avian pathology (7 of the 19 avian strains).

Few data are available on this clonal group from poultry. Recently, Vincent et al. (41) first reported on the presence of one O25b:H4-ST131 isolate from among 250 retail chicken samples (0.4%) with a macrorestriction profile indistinguishable from that of an *E. coli* strain originating from a human UTI. That O25b:H4-ST131 strain found by Vincent et al. (41) was negative for ESBL production. Before that, Carattoli (6) gathered in her review two reports of CTX-M-9-producing *E. coli* in poultry but did not detail the serotypes or STs.

According to Johnson et al. (20), it seems that clone ST131 is common among *E. coli* strains resistant to FQs and does not necessarily have to produce an ESBL. In agreement with this, Peirano and Pitout (35) believe that plasmids carrying CTX-M-15 enzymes were most likely introduced at a later stage and that ST131 was possibly an established successful FQ-resistant clone before it acquired plasmids encoding CTX-M-15. On the basis of our data, it seems that not only plasmids encoding CTX-M-15 but also plasmids encoding other CTX-M types were probably introduced at a later stage. In fact, it has not been until recently that we first detected the CTX-M-9 enzyme in human strains belonging to clonal group O25b:H4-ST131 in our sanitary area (first isolation in 2008). A study of the prevalence of 761 ESBL-producing *E. coli* isolates recovered from 2006 to 2009 from patients admitted to five hospitals in Galicia (northwest Spain) showed that 143 (18.8%) belonged to the O25b:H4-ST131 clonal group and produced different CTX-M types: 128 of those 143 strains produced CTX-M-15 (89.5%), 6 produced CTX-M-14 (4.2%), 5 produced CTX-M-9 (3.5%), 3 produced CTX-M-32 (2.1%), and 1 produced CTX-M-1 (0.7%) (unpublished data). Those 5 CTX-M-9-producing human strains (all *ibeA* positive) were compared in the present study with the 19 avian isolates. Interestingly, three of the five CTX-M-9-producing human strains clustered (89.6% similarity) with six avian strains producing the same type of enzyme and showing the same virulence profile (P3-11; Fig. 2). These three human CTX-M-9-producing strains harbored the K1 capsule antigen, a virulence factor statistically linked to strains of avian origin (Table 2). Furthermore, the macrorestriction profiles of the nine P3-11 strains producing CTX-M-9 included in this cluster clearly differed in similarity from the remaining 15 O25b:H4-ST131 human strains producing CTX-M enzymes other than CTX-M-9, none of which was *ibeA* positive (Fig. 2). The high XbaI macrorestriction similarity shared by the nine CTX-M-9-producing avian and human strains would be indic-

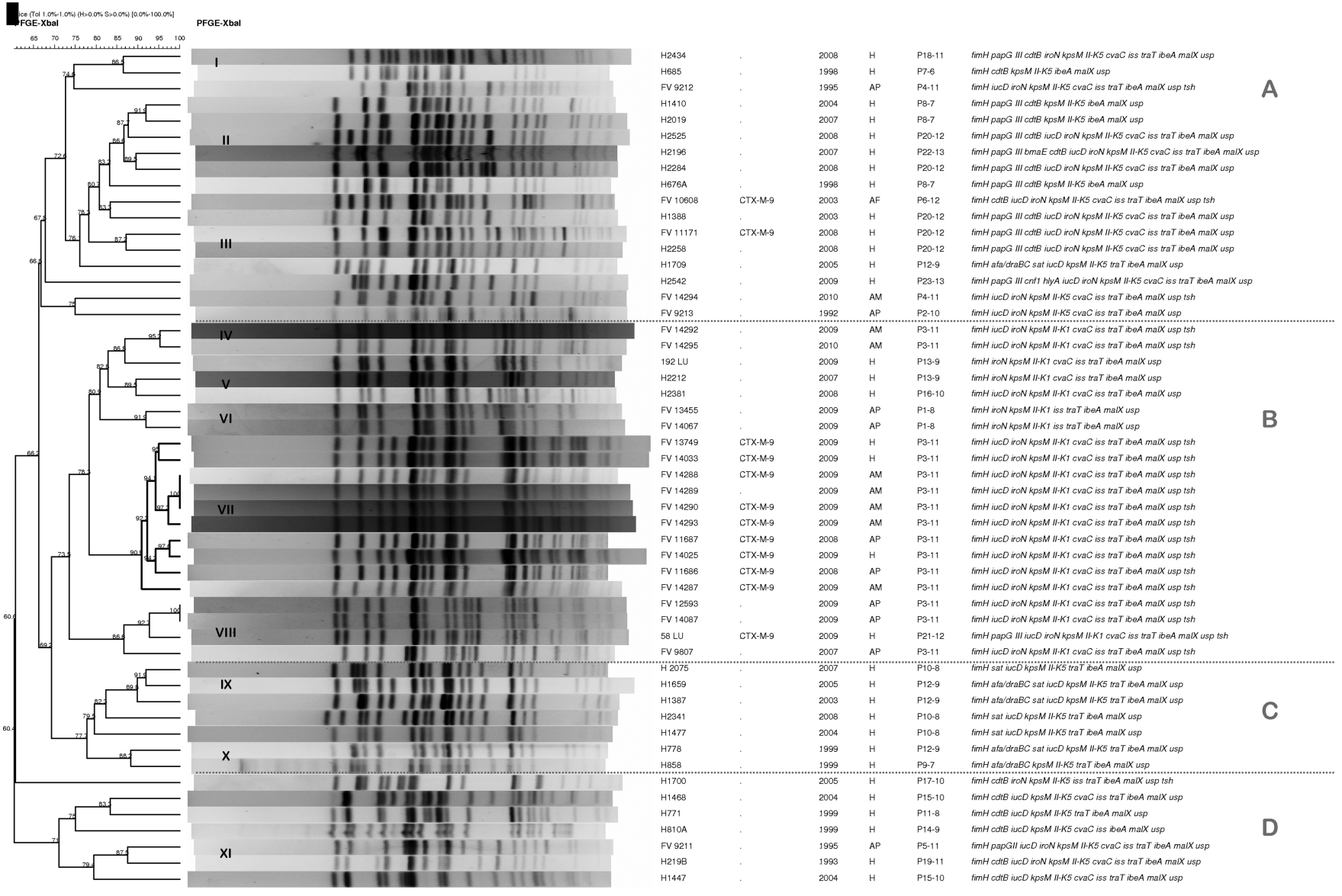

FIG. 1. Dendrogram of XbaI macrorestriction profiles obtained by PFGE of the 19 avian and 33 human O25b:H4-ST131 *ibeA*-positive strains characterized in this study. The dendrogram was generated by use of the UPGMA algorithm, based on the Dice similarity coefficient with a 1.0% band position tolerance. The strain code, ESBL type, year and origin (H, human; AP, avian pathology associated; AM, avian meat) of isolation, virulence profile designation-number of virulence factors, and virulence factors are shown on the right-hand side.

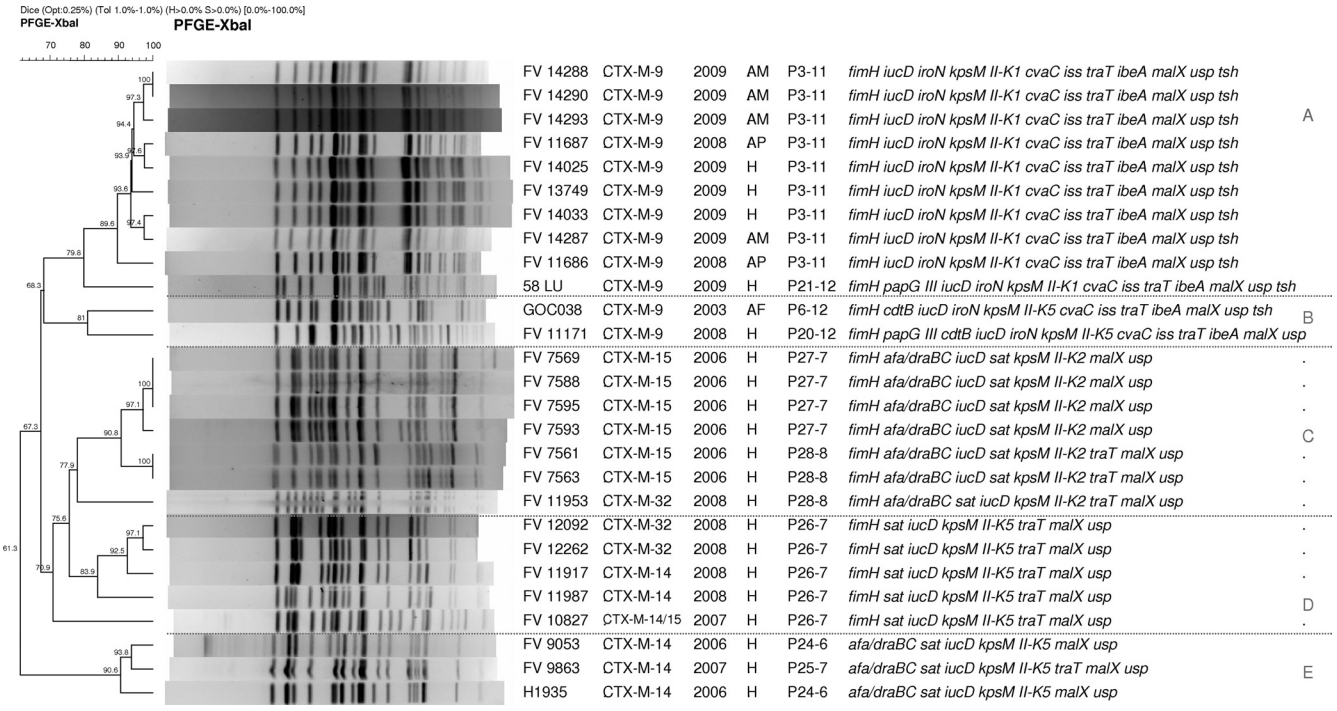

FIG. 2. Dendrogram of XbaI macrorestriction profiles obtained by PFGE of the 12 CTX-M-9-producing O25b:H4-ST131 strains (7 avian and 5 human strains) characterized in this study by comparison with 15 human O25b:H4-ST131 *E. coli* strains producing different types of CTX-M enzymes (6 strains producing CTX-M-15, 5 producing CTX-M-14, 3 producing CTX-M-32, and 1 producing CTX-M-14/15). The dendrogram was generated by use of the UPGMA algorithm, based on the Dice similarity coefficient with a 1.0% band position tolerance. The strain code, ESBL type, year and origin (H, human; AP, avian pathology associated; AM, avian meat) of isolation, virulence profile designation-number of virulence factors, and virulence factors are shown on the right-hand side.

ative of recent emergence and circulation between both hosts, as molecular typing by PFGE shows that small changes happened and quickly accumulated in the genome (local epidemiology). As far as we know, this is the first study that clearly reports on the zoonotic potential of avian strains belonging to clonal group O25b:H4-ST131 and producing CTX-M enzymes.

Peirano and Pitout (35) found that the virulence factors *malX*, *ompT*, and *usp* were more common in ST131 strains than in other successful clonal groups (O15 and clonal group A). These authors suggest that the cited genes might be important in the worldwide dissemination of clonal group ST131. We did not analyze the isolates for the presence of *ompT*, but all 67 human and avian O25b:H4-ST131 strains included in the present study were positive for *usp* and *malX*.

Forty-six of the 52 O25b:H4-ST131 *ibeA*-positive *E. coli* strains (29 human and 17 avian strains) satisfied the criteria for extraintestinal pathogenic status. Besides, all 52 *ibeA*-positive strains showed a high pathogenic potential, according to the number of virulence genes harbored (mean, 10.1; range, 6 to 13). Interestingly, all 19 avian strains of the present study harbored the *ibeA* gene, which has clearly been linked to the pathogenicity of ExPEC strains and which is positively associated with serogroups O2, O18, and O88 (1, 14).

In conclusion, the O25b:H4-ST131 *ibeA*-positive clonal group of *E. coli* has recently emerged among avian isolates. These strains have newly acquired the K1 capsule antigen and include CTX-M-9-producing strains. This clonal group represents a real zoonotic risk that has crossed the barrier between human and avian hosts, since several PFGE clusters (>85% similarity) identified in this study included both human and avian isolates. The most prominent of these clusters was the one with 10 strains showing the same virulence-gene profile, of which 9 were CTX-M-9-producing strains.

ACKNOWLEDGMENTS

This work was partially supported by grants REIPI RD06/0008/1016-1018 and PS09/01273 (Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III, Fondo de Investigación Sanitaria), AGL-2008-02129 (Ministerio de Ciencia e Innovación), and 09TAL007261PR and 2007/000044-0 (Xunta de Galicia and the European Regional Development Fund [ERDF]). Azucena Mora acknowledges financial support from the Ramón y Cajal program of the Spanish Ministerio de Ciencia e Innovación. Rosalia Mamani acknowledges a grant from the Agencia Española de Cooperación Internacional (AECI) (Ministerio de Asuntos Exteriores y de Cooperación).

We thank Monserrat Lamela and Angeles Espiño for skillful technical assistance.

REFERENCES

1. **Bidet, P., F. Mahjoub-Messai, J. Blanco, J. Blanco, M. Dehem, Y. Aujard, E. Bingen, and S. Bonacorsi.** 2007. Combined multilocus sequence typing and O serogrouping distinguishes Escherichia coli subtypes associated with infant urosepsis and/or meningitis. J. Infect. Dis. **196:**297–303.
2. **Blanco, J., M. P. Alonso, E. A. González, M. Blanco, and J. I. Garabal.** 1990. Virulence factors of bacteraemic Escherichia coli with particular reference to production of cytotoxic necrotising factor (CNF) by P-fimbriate strains. J. Med. Microbiol. **31:**175–183.
3. **Blanco, J. E., M. Blanco, A. Mora, W. H. Jansen, V. García, M. L. Vázquez, and J. Blanco.** 1998. Serotypes of Escherichia coli isolated from septicaemic chickens in Galicia (northwest Spain). Vet. Microbiol. **61:**229–235.
4. **Blanco, M., M. P. Alonso, M. H. Nicolas-Chanoine, G. Dahbi, A. Mora, J. E.**

Blanco, C. López, P. Cortés, M. Llagostera, V. Leflon-Guibout, B. Puentes, R. Mamani, A. Herrera, M. A. Coira, F. García-Garrote, J. M. Pita, and J. Blanco. 2009. Molecular epidemiology of Escherichia coli producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b:H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother. **63:**1135–1141.

5. **Cagnacci, S., L. Gualco, E. Debbia, G. C. Schito, and A. Marchese.** 2008. European emergence of ciprofloxacin-resistant *Escherichia coli* clonal groups O25:H4-ST 131 and O15:K52:H1 causing community-acquired uncomplicated cystitis. J. Clin. Microbiol. **46:**2605–2612.
6. **Carattoli, A.** 2008. Animal reservoirs for extended spectrum beta-lactamase producers. Clin. Microbiol. Infect. **14**(Suppl. 1)**:**117–123.
7. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
8. **Clermont, O., H. Dhanji, M. Upton, T. Gibreel, A. Fox, D. Boyd, M. R. Mulvey, P. Nordmann, E. Ruppé, J. L. Sarthou, T. Frank, S. Vimont, G. Arlet, C. Branger, N. Woodford, and E. Denamur.** 2009. Rapid detection of the O25b-ST131 clone of Escherichia coli encompassing the CTX-M-15-producing strains. J. Antimicrob. Chemother. **64:**274–277.
9. **Coque, T. M., A. Novais, A. Carattoli, L. Poirel, J. Pitout, L. Peixe, F. Baquero, R. Cantón, and P. Nordmann.** 2008. Dissemination of clonally related Escherichia coli strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg. Infect. Dis. **14:**195–200.
10. **Cortés, P., V. Blanc, A. Mora, G. Dahbi, J. E. Blanco, M. Blanco, C. López, A. Andreu, F. Navarro, M. P. Alonso, G. Bou, J. Blanco, and M. Llagostera.** 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl. Environ. Microbiol. **76:**2799–2805.
11. **Dho-Moulin, M., and J. M. Fairbrother.** 1999. Avian pathogenic Escherichia coli (APEC). Vet. Res. **30:**299–316.
12. **Ewers, C., G. Li, H. Wilking, S. Kiessling, K. Alt, E. M. Antáo, C. Laturnus, I. Diehl, S. Glodde, T. Homeier, U. Böhnke, H. Steinrück, H. C. Philipp, and L. H. Wieler.** 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: how closely related are they? Int. J. Med. Microbiol. **297:**163–176.
13. **Ewers, C., M. Grobbel, I. Stamm, P. A. Kopp, I. Diehl, T. Semmler, A. Fruth, J. Beutlich, B. Guerra, L. H. Wieler, and S. Guenther.** 2010. Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing Escherichia coli among companion animals. J. Antimicrob. Chemother. **65:**651–660.
14. **Germon, P., Y. H. Chen, L. He, J. E. Blanco, A. Brée, C. Schouler, S. H. Huang, and M. Moulin-Schouleur.** 2005. ibeA, a virulence factor of avian pathogenic Escherichia coli. Microbiology **51:**1179–1186.
15. **Guinée, P. A. M., W. H. Jansen, T. Wadström, and R. Sellwood.** 1981. Escherichia coli associated with neonatal diarrhoea in piglets and calves, p. 126–162. *In* P. W. Leeww and P. A. M. Guinée (ed.), Laboratory diagnosis in neonatal calf and pig diarrhoea. Current topics in veterinary and animal science. Martinus-Nijhoff, The Hague, Netherlands.
16. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
17. **Johnson, J. R., A. C. Murray, A. Gajewski, M. Sullivan, P. Snippes, M. A. Kuskowski, and K. E. Smith.** 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. **47:**2161–2168.
18. **Johnson, J. R., and T. A. Russo.** 2005. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) Escherichia coli. Int. J. Med. Microbiol. **295:**383–404.
19. **Johnson, J. R., J. S. McCabe, D. G. White, B. Johnston, M. A. Kuskowski, and P. McDermott.** 2009. Molecular analysis of Escherichia coli from retail meats (2002–2004) from the United States National Antimicrobial Resistance Monitoring System. Clin. Infect. Dis. **49:**195–201.
20. **Johnson, J. R., M. Menard, B. Johnston, M. A. Kuskowski, K. Nichol, and G. G. Zhanel.** 2009. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob. Agents Chemother. **53:**2733–2739.
21. **Johnson, J. R., S. Miller, B. Johnston, C. Clabots, and C. Debroy.** 2009. Sharing of *Escherichia coli* sequence type ST131 and other multidrug-resistant and urovirulent *E. coli* strains among dogs and cats within a household. J. Clin. Microbiol. **47:**3721–3725.
22. **Johnson, J. R., B. Johnston, C. Clabots, M. A. Kuskowski, S. Pendyala, C. Debroy, B. Nowicki, and J. Rice.** 2010. *Escherichia coli* sequence type ST131 as an emerging fluoroquinolone-resistant uropathogen among renal transplant recipients. Antimicrob. Agents Chemother. **54:**546–550.
23. **Kim, K. S.** 2002. Strategy of Escherichia coli for crossing the blood-brain barrier, J. Infect. Dis. **186**(Suppl. 2)**:**S220–S224.
24. **Kim, K. J., S. J. Elliott, F. Di Cello, M. F. Stins, and K. S. Kim.** 2003. The K1 capsule modulates trafficking of E. coli-containing vacuoles and enhances intracellular bacterial survival in human brain microvascular endothelial cells. Cell. Microbiol. **5:**245–252.
25. **Leflon-Guibout, V., C. Jurand, S. Bonacorsi, F. Espinasse, M. C. Guelfi, F. Duportail, B. Heym, E. Bingen, and M. H. Nicolas-Chanoine.** 2004. Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob. Agents Chemother. **48:**3736–3742.
26. **Leflon-Guibout, V., J. Blanco, K. Amaqdouf, A. Mora, L. Guize, and M. H. Nicolas-Chanoine.** 2008. Absence of CTX-M enzymes but high prevalence of clones, including clone ST131, among fecal *Escherichia coli* isolates from healthy subjects living in the area of Paris, France. J. Clin. Microbiol. **46:** 3900–3905.
27. **Manges, A. R., H. Tabor, P. Tellis, C. Vincent, and P. P. Tellier.** 2008. Endemic and epidemic lineages of Escherichia coli that cause urinary tract infections. Emerg. Infect. Dis. **14:**1575–1583.
28. **Martinez-Medina, M., A. Mora, M. Blanco, C. López, M. P. Alonso, S. Bonacorsi, M. H. Nicolas-Chanoine, A. Darfeuille-Michaud, J. Garcia-Gil, and J. Blanco.** 2009. Similarity and divergence among adherent-invasive *Escherichia coli* and extraintestinal pathogenic E. coli strains. J. Clin. Microbiol. **47:**3968–3979.
29. **Mora, A., C. López, G. Dabhi, M. Blanco, J. E. Blanco, M. P. Alonso, A. Herrera, R. Mamani, S. Bonacorsi, M. Moulin-Schouleur, and J. Blanco.** 2009. Extraintestinal pathogenic Escherichia coli O1:K1:H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. BMC Microbiol. **9:**132.
30. **Moulin-Schouleur, M., C. Schouler, P. Tailliez, M. R. Kao, A. Brée, P. Germon, E. Oswald, J. Mainil, M. Blanco, and J. Blanco.** 2006. Common virulence factors and genetic relationships between O18:K1:H7 *Escherichia coli* isolates of human and avian origin. J. Clin. Microbiol. **44:**3484–3492.
31. **Moulin-Schouleur, M., M. Répérant, S. Laurent, A. Brée, S. Mignon-Grasteau, P. Germon, D. Rasschaert, and C. Schouler.** 2007. Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. J. Clin. Microbiol. **45:**3366–3376.
32. **NCCLS/CLSI.** 2008. Performance standards for antimicrobial disc susceptibility testing: 18th informational supplement. M100-S18. NCCLS/CLSI, Wayne, PA.
33. **Nicolas-Chanoine, M. H., J. Blanco, V. Leflond-Guibout, R. Demarty, M. P. Alonso, M. M. Caniça, Y. J. Park, J. P. Lavigne, J. Pitout, and J. R. Johnson.** 2008. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother. **61:**273–281.
34. **Oteo, J., K. Diestra, C. Juan, V. Bautista, A. Novais, M. Pérez-Vázquez, B. Moyá, E. Miró, T. M. Coque, A. Oliver, R. Cantón, F. Navarro, J. Campos, and the Spanish Network in Infectious Pathology Project (REIPI).** 2009. Extended-spectrum beta-lactamase-producing Escherichia coli in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Int. J. Antimicrob. Agents **34:** 173–176.
35. **Peirano, G., and J. D. Pitout.** 2010. Molecular epidemiology of Escherichia coli producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. Int. J. Antimicrob. Agents **35:**316–321.
36. **Pitout, J. D., D. B. Gregson, L. Campbell, and K. B. Laupland.** 2009. Molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. Antimicrob. Agents Chemother. **53:**2846–2851.
37. **Pomba, C., J. D. da Fonseca, B. C. Baptista, J. D. Correia, L. Martínez-Martínez.** 2009. Detection of the pandemic O25-ST131 human virulent *Escherichia coli* CTX-M-15-producing clone harboring the *qnrB2* and *aac(6′)-Ib-cr* genes in a dog. Antimicrob. Agents Chemother. **53:**327–328.
38. **Stordeur, P., D. Marlier, J. Blanco, E. Oswald, F. Biet, M. Dho-Moulin, and J. Mainil.** 2002. Examination of Escherichia coli from poultry for selected adhesin genes important in disease caused by mammalian pathogenic E. coli. Vet. Microbiol. **84:**231–241.
39. **Suzuki, S., N. Shibata, K. Yamane, J. Wachino, K. Ito, and Y. Arakawa.** 2009. Change in the prevalence of extended-spectrum-beta-lactamase-producing Escherichia coli in Japan by clonal spread. J. Antimicrob. Chemother. **63:**72–79.
40. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
41. **Vincent, C., P. Boerlin, D. Daignault, C. M. Dozois, L. Dutil, C. Galanakis, R. J. Reid-Smith, P. P. Tellier, P. A. Tellis, K. Ziebell, and A. R. Manges.** 2010. Food reservoir for Escherichia coli causing urinary tract infections. Emerg. Infect. Dis. **16:**88–95.