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# Phylogenetic Grouping and Virulence Potential of Extended-Spectrum- $\beta$ -Lactamase-Producing *Escherichia coli* Strains in Cattle

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**In line with recent reports of extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* isolates of highly virulent serotypes, such as O104:H4, we investigated the distribution of phylogroups (A, B1, B2, D) and virulence factor (VF)-encoding genes in 204 ESBL-producing *E. coli* isolates from diarrheic cattle. ESBL genes, VFs, and phylogroups were identified by PCR and a commercial DNA array (Alere, France). ESBL genes belonged mostly to the CTX-M-1 (65.7%) and CTX-M-9 (27.0%) groups, whereas those of the CTX-M-2 and TEM groups were much less represented (3.9% and 3.4%, respectively). One ESBL isolate was *stx*<sub>1</sub> and *eae* positive and belonged to a major enterohemorrhagic *E. coli* (EHEC) serotype (O111:H8). Two other isolates were *eae* positive but *stx* negative; one of these had serotype O26:H11. ESBL isolates belonged mainly to phylogroup A (55.4%) and, to lesser extents, to phylogroups D (25.5%) and B1 (15.6%), whereas B2 strains were quasi-absent (1/204). The number of VFs was significantly higher in phylogroup B1 than in phylogroups A ( $P = 0.04$ ) and D ( $P = 0.02$ ). Almost all of the VFs detected were found in CTX-M-1 isolates, whereas only 64.3% and 33.3% of them were found in CTX-M-9 and CTX-M-2 isolates, respectively. These results indicated that the widespread dissemination of the *bla*<sub>CTX-M</sub> genes within the *E. coli* population from cattle still spared the subpopulation of EHEC/Shiga-toxigenic *E. coli* (STEC) isolates. In contrast to other reports on non-ESBL-producing isolates from domestic animals, B1 was not the main phylogroup identified. However, B1 was found to be the most virulent phylogroup, suggesting host-specific distribution of virulence determinants among phylogenetic groups.**

Extended-spectrum beta-lactamases (ESBLs) are a group of enzymes mediating resistance to most beta-lactams used in human and veterinary medicine, including expanded-spectrum cephalosporins but excluding carbapenems and cephamycins (14). These enzymes are now widely distributed worldwide in Gram-negative bacteria (particularly in *Enterobacteriaceae*), with a specific and still growing expansion of those of the CTX-M type (8, 25). The high prevalence and distribution of the *bla*<sub>CTX-M</sub> genes has been shown to be related to their ability to spread horizontally among different lineages of bacteria as well as vertically in association with successful clones (34).

The great prevalence of the *bla*<sub>CTX-M-15</sub> gene worldwide is a significant example of such vertical spread, since this gene disseminated successfully in humans, mostly in association with the highly virulent O25b:H4-ST131 extraintestinal pathogenic *Escherichia coli* (ExPEC) clone (35, 38, 41). In contrast to ExPEC, where virulence traits and ESBL genes within the same *E. coli* strain have been reported regularly, the common enterohemorrhagic *E. coli* (EHEC) serotypes were rarely associated with ESBLs (7, 17, 20, 40). Very recently, the *E. coli* O104:H4 outbreak in Europe was also caused by a highly aggressive *E. coli* strain that had acquired resistance to expanded-spectrum cephalosporins (4, 30). In this *E. coli* clone, a *bla*<sub>CTX-M-15</sub> gene was carried on an IncI1-type plasmid of clonal complex 31 (CC31), an association also recently identified by our group in clinical *E. coli* isolates from cattle (27). This demonstrates that the same pool of *bla*<sub>CTX-M-15</sub>-carrying plasmids is shared by *E. coli* isolates from cattle and EHEC strains that are pathogenic to humans.

Moreover, we recently reported the first *bla*<sub>CTX-M-15</sub> gene in a Shiga toxin-producing *E. coli* (STEC) isolate of serotype O111:H8 (47), again recovered from cattle, which are also recognized as the main EHEC reservoir (9). This prompted us to study a large col-

lection of 204 ESBL-producing *E. coli* isolates from cattle in order to investigate to what extent ESBL genes may have invaded the subpopulation of EHEC strains potentially pathogenic to humans. In particular, we aimed to identify the phylogroups and serotypes of those strains and to determine the distribution of the main virulence factors.

## MATERIALS AND METHODS

**Bacterial strains.** A total of 204 ESBL-producing *E. coli* strains isolated from 2006 to 2010 were included in this study. Each sample was nonreplicate, meaning that only one sample per farm was included in this study. Isolates representing the dominant bacterial flora were recovered from specimens of sick (diarrhea [ $n = 182$ ], septicemia [ $n = 2$ ], or undefined illness [ $n = 4$ ]) or dead ( $n = 16$ ) calves collected from 39 different geographic areas (districts) through the RESAPATH Network, which carries out surveillance of antimicrobial resistance in pathogens causing animal infections in France ([www.resapath.anses.fr](http://www.resapath.anses.fr)). Isolates were identified using conventional methods, i.e., colony morphology and API 20E tests (bioMérieux, Marcy l'Etoile, France). Susceptibility to 32  $\beta$ -lactam and non- $\beta$ -lactam antimicrobials was tested by the standard disc diffusion method according to CLSI standards (12). *E. coli* strain ATCC 25922 was used for quality control. ESBL production was determined by a standard double-disc synergy test.

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TABLE 1 Primers and probes used for detection of ESBL-producing isolates and *E. coli* virulence-associated genes

Primer designation	Primer sequence <sup>a</sup>	Target	Size of PCR product (bp)	Reference
Stx1F	5'-GACTGCAAAGACGTATGTAGATTTCG-3'	<i>stx</i> <sub>1</sub>	150	16
Stx1R	5'-ATCTATCCCTCTGACATCAACTGC-3'			
SLT12	5'-ATCCTATTCCCGGAGTTTACG-3'	<i>stx</i> <sub>2</sub>	584	10
SLT13	5'-GCGTCATCGTATACACAGGAGC-3'			
eae.f	5'-GAACGGCAGAGGTTAATCTG-3'	<i>eae</i>	203	11
eae.r	5'-CAATGAAGACGTTATAGCCC-3'			
EspPF	5'-AAACAGCAGGCACTTGAACG-3'	<i>espP</i>	1,830	22
EspPR	5'-GGAGTCGTCAGTCAGTAGAT-3'			
HlyAF	5'-GCATCATCAAGCGTACGTTCC-3'	$\alpha$ - <i>hlyA</i>	534	37
HlyAR	5'-AATGAGCCAAGCTGGTTAAGCT-3'			
FSH	5'-GAGAATATCGGTACCGCCATGTTTGC-3'	<i>msbB2</i>	715	18
R4	5'-TATTGCTGGGTGAGCTCATTATCCTG-3'			
ISEcp1L1	5'-CAGCTTTTATGACTCG-3'	CTX-M group 1	1,100	24
P2D	5'-CAGCGCTTTTCCCGTCTAAG-3'			
Tem_F	5'-ATAAAATTCTGAAGACGAAA-3'	TEM	1,080	21
Tem_R	5'-GACAGTTACCAATGCTTAATC-3'			
pagC.f	5'-ACCCACACTGTTTCTCCACTCA-3'	<i>pagC</i> <sup>b</sup>	89	47
pagC.r	5'-GTCTGAATGACTATATCAGTTTTTATGGTTTG-3'			
pagC.p	5'-FAM-AATCTCCCTTCGCTTTAGTATGAGATATCCCCA-BHQ1-3'			
nleB.f	5'-CCCTGCCAGTGAGAGGGATA-3'	<i>nleB</i> <sup>b</sup>	81	47
nleB.r	5'-AAAGAGTCCTTACCTTCTGGGATATTT-3'			
nleB.p	5'-HEX-ITATCTGTTAGGCTTATTAAGAGAAGAGTT-BHQ2-3'			
efa1.f	5'-TTCATCATAACAGGTAAGTAAATCAT-3'	<i>efa1</i> <sup>b</sup>	82	47
efa1.r	5'-TCCCGTAAGCCATTATAAACATTTG-3'			
efa1.p	5'-Cy5-ATTGTTACACAACGCGCTCCTTGGTCTG-BHQ2-3'			

<sup>a</sup> FAM, 6-carboxyfluorescein; BHQ, black hole quencher.

<sup>b</sup> Gene harbored on O island 122.

**ESBL genes, phylogrouping, and determination of the main O:H serotypes.** DNA was extracted and purified using the DNeasy Blood and Tissue kit (Qiagen, France). The *bla*<sub>CTX-M</sub> genes were detected using a CTX-M group-specific multiplex PCR (42). In order to identify possible CTX-M-15 producers, which are often associated with pathogenicity to humans, an additional PCR using the external primers ISEcp1L1 and P2D (Table 1) was performed on all CTX-M group 1 isolates that were resistant to ceftazidime by diffusion, and the amplicon was sequenced. ESBL genes other than the *bla*<sub>CTX-M</sub> genes were checked by PCR for the *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, and *bla*<sub>TEM</sub> genes (31, 32).

The major phylogenetic groups (A, B1, B2, and D) were assigned as described previously by Clermont et al. (11). Finally, the five major EHEC serogroups that are pathogenic to humans (O26, O111, O103, O145, and O157) (15) were searched by PCR as described previously (33, 37), and positive isolates were further characterized for their H flagellar antigens by real-time PCR as described previously (28).

**Virulence determinants.** On all ESBL isolates, a first screening was carried out by individual PCRs for the presence of *stx*<sub>1</sub>, *stx*<sub>2</sub>, and *eae* virulence genes and the plasmid-encoded *espP* gene, as follows. The *espP*, *eae*, *stx*<sub>1</sub>, and *stx*<sub>2</sub> genes were detected as described previously (10, 13, 22) by using the primers listed in Table 1. *eae* variants and the genomic island OI-122 were detected by real-time PCR (29, 47). A subset of 36 *E. coli* isolates from the A (13 isolates), B1 (10 isolates), and D (13 isolates) phylogroups that were positive for the *eae*, *stx*<sub>1</sub>, and/or *espP* gene was randomly sampled and was subjected to DNA microarray analysis (Identibac EC, Alere, France) for the presence of *stx*<sub>1</sub>, *stx*<sub>2</sub>, *eae*, and 38 additional virulence factors (VFs) (see Table 4). PCR amplification, labeling, and hybridization steps were performed according to the manufacturers' instructions and previously described protocols (2).

**Statistical analysis.** Proportions were compared by using the chi-square test in the Epi Info software. To assess the effects of both phylogenetic and CTX-M groups on the number of VFs detected by DNA array,

analyses of variance (ANOVA) were performed using R software. The threshold for statistical significance was a *P* value below 0.05.

## RESULTS

**Determination of ESBL groups, other resistances, and phylogroups.** Among the 204 ESBL-producing *E. coli* strains, 134 produced a CTX-M group 1 enzyme (65.7%), 8 produced a CTX-M group 2 enzyme (4.0%), and 55 produced a CTX-M group 9 enzyme (26.9%) (Table 2). Seven (3.4%) ESBL phenotypes were due to *bla*<sub>TEM-52</sub> ESBL genes. Among the 134 CTX-M-1-producing isolates, 22 (16.4%) were resistant to ceftazidime and were confirmed to produce a CTX-M-15 enzyme after full sequencing of the CTX-M group 1 amplicon.

All ESBL-producing isolates were allocated to one of the four phylogenetic groups A, B1, B2, and D. The results showed that 55.4% (113/204) of these isolates belonged to group A, 25.5% (52/204) to group D, and 15.6% (32/204) to group B1 (*P*, <0.05 for A versus B1 versus D) (Table 2). Only one strain was found in group B2. No significant differences in the distributions of the CTX-M-2 and CTX-M-9 groups were observed between phylogroups (*P*, >0.05 for A versus B1 versus D) (Table 2). It is noteworthy that a very limited proportion of all ESBL-producing *E. coli* isolates (5/204 [2.5%]) were resistant to beta-lactams only. In general, elevated proportions of coresistance were observed: 180/204 (88.2%), 183/204 (89.7%), or 141/204 (69.1%) isolates were resistant to aminoglycosides, tetracyclines, or fluoroquinolones, respectively (Table 3).

**Virulence factors of the ESBL-producing *E. coli* isolates.** Among the 204 ESBL-producing *E. coli* strains, 3 (1.5%) isolates

TABLE 2 Distribution of ESBL types according to phylogenetic groups

Phylogenetic group	No. (%) of isolates <sup>a</sup> producing:				Total no. (% of all isolates)
	CTX-M group 1	CTX-M group 2	CTX-M group 9	TEM <sub>ESBL</sub>	
A	77 (37.7)	4 (2.0)	28 (13.7)	4 (2.0)	113 (55.4)*
B1	22 (10.8)	1 (0.5)	7 (3.4)	2 (0.9)	32 (15.6)
B2	1 (0.5)	0 (0)	0 (0)	0 (0)	1 (0.5)
D	29 (14.2)	3 (1.5)	19 (9.3)	1 (0.5)	52 (25.5)
ND <sup>b</sup>	5 (2.5)	0 (0)	1 (0.5)	0 (0)	6 (3.0)
Total	134 (65.7)	8 (4.0)	55 (26.9)	7 (3.4)	204 (100)

<sup>a</sup> Proportions ( $n > 5$ ) for different phylogroups are compared. An asterisk indicates a global  $P$  value of  $\leq 0.05$  by the chi-square test.

<sup>b</sup> ND, not determined.

were *eae* positive (Table 4). In addition to the O111:H8 (*stx*<sub>1</sub>-positive) isolate reported previously (47), only one isolate belonged to one of the five main EHEC serogroups (i.e., O157, O26, O111, O103, and O145) that are pathogenic to humans (15). This isolate was further identified as belonging to the O26:H11 serotype and the B1 phylogroup. This strain carried the *bla*<sub>CTX-M-9</sub> gene and harbored the *eae*  $\beta$ 1 variant and OI-122 but was *stx* negative. The O26:H11 isolate was confirmed again as *stx* negative by DNA array and was also found to carry the *tox*B gene, which encodes an EHEC-associated adhesin. Fifty-seven of 204 strains (28%) were *esp*P positive.

Interestingly, among the subset of 36 isolates that were tested by microarray, the number of VFs detected differed by phylogroup; it was significantly higher in group B1 than in group A ( $P$ , 0.04 by ANOVA) and group D ( $P$ , 0.02 by ANOVA) (Table 4). The same phenomenon was highlighted by using the proportion of VF recovery in the different phylogroups (number of VFs detected in the group among all the VFs detected in the 36 tested strains) ( $P$ , 0.03 for A versus B1 versus D) (Table 4). These VFs include the intimin and type III secretion system (T3SS) effectors (Cif, Esp [EspB, EspF, EspJ], Nle [NleA, NleB, NleC], Tir, TccP), adhesins (FanA, Fim41, Efa1, ToxB), hemolysin ( $\alpha$ -HlyA), toxins (Stx1), and the plasmid-encoded catalase KatP, all of which were associated with phylogroup B1 only (Table 4). Whereas these highly virulent genes were associated with the B1 phylogroup, others, such as adhesins (IpfA, PrfB/PapB, Iha, F17, H), bacteriocin (Mch), and siderophore receptors (IreA), were distributed mainly in phylogroups A and D (Table 4). In each of phylogroups A and

D, only 1 isolate among the 13 isolates tested did not carry any gene encoding fimbriae or other adhesins.

The adhesins PrfB and Iha, detected mostly in human commensal and clinical *E. coli* strains (44), were more prevalent than fimbriae and were distributed equally among phylogroups (Table 4). Interestingly, genes coding for F41 fimbriae (*fim41a*, *fanA*) were linked to phylogroup B1, and those coding for F17 fimbriae were found in phylogroup A only. The gene encoding long polar fimbriae (*ipfA*), which characterizes a common virulence group statistically associated with human diarrheal disease (1), was equally distributed among phylogroups. Also, the *astA* gene, coding for the EAST1 toxin, found mostly in EHEC and enteroaggregative *E. coli* (EaggEC) strains (36) but negatively associated with severe human diarrhea (1), was significantly linked with phylogroup A in this study.

The single isolate of the B2 phylogroup carried the *iss*, *mchH*, and *ipfA* genes (data not shown), which were also found in phylogroups A, B1, and D. Some other VF genes specific to the enterotoxigenic *E. coli* (ETEC) pathotype, such as *cfaC*, *fasA*, *lthA*, and *stb*, as well as *cnf* and *sfaS*, found mainly in ExPEC (2) and in group B2 (1), remained absent in this collection.

The proportions of recovery of the VFs in the different CTX-M groups were significantly different ( $P$ , 0.007 for CTX-M-1 versus CTX-M-2 versus CTX-M-9): almost all of the VFs detected (92.9%) were found in CTX-M-1 isolates, whereas only 64.3% and 33.3% of them were found in CTX-M-9 and CTX-M-2 isolates, respectively (Table 4). However, no statistical difference between the numbers of VFs within CTX-M groups was identified.

## DISCUSSION

In animals, ESBLs have become widely disseminated among *E. coli* isolates. However, little is known about how ESBL genes are distributed within *E. coli* isolates that are pathogenic to animals versus those that are part of their commensal flora. For humans, ESBL genes have also been widely reported in ExPEC, in contrast to EHEC, isolates. Interestingly, we reported the first *bla*<sub>CTX-M-15</sub> gene in a Shiga toxin-producing *E. coli* isolate of serotype O111:H8 recovered from cattle (47), which are also recognized as the main STEC/EHEC reservoir. Thus, the aim of this work was to further estimate the proportion of STEC/EHEC strains potentially highly pathogenic to humans in a very large collection of ESBL-producing *E. coli* strains from cattle.

To the best of our knowledge, this is the largest collection of ESBL-producing *E. coli* strains from cattle reported so far. We showed that ESBLs of the CTX-M-1 group, followed by those of the CTX-M-9 group, are predominant in diseased cattle, and par-

TABLE 3 Resistance associated with  $\beta$ -lactams in *E. coli* isolates

Co-resistance	No. (%) of strains
Aminoglycosides <sup>a</sup>	180 (88.2)
Phenicol <sup>b</sup>	125 (61.3)
Tetracyclines	183 (89.7)
Quinolones <sup>c</sup>	149 (73.0)
Fluoroquinolones <sup>d</sup>	141 (69.1)
Sulfonamides-trimethoprim <sup>e</sup>	150 (73.5)

<sup>a</sup> Including streptomycin (175/204 isolates [85.8%]), kanamycin (144/204 [70.6%]), tobramycin (75/204 [36.8%]), and gentamicin (71/204 [34.8%]).

<sup>b</sup> Including chloramphenicol (126/204 isolates [61.8%]) and florfenicol (64/204 [31.4%]).

<sup>c</sup> Nalidixic acid was tested.

<sup>d</sup> Enrofloxacin was tested.

<sup>e</sup> In addition to the strains resistant to the combination, 41/204 (20.1%) strains were resistant to sulfonamides only and 2/204 (1.0%) strains were resistant to trimethoprim only.

TABLE 4 Distribution of virulence genes detected by microarray in ESBL-producing *E. coli* isolates according to phylogroups and CTX-M groups<sup>a</sup>

VF function	Gene	No. of isolates in:					
		Phylogroup:			CTX-M group <sup>b</sup> :		
		A (n = 13)	B1 (n = 10)	D (n = 13)	1 (n = 18)	2 (n = 4)	9 (n = 13)
Serum survival	<i>iss</i>	8	5	8	10	3	7
Intimin	<i>eae</i>	0	3	0	2	0	1
T3SS and effectors	<i>espA</i>	1	3	1	2	0	3
	<i>espB</i>	0	1	0	0	0	1
	<i>espF</i>	0	3	0	2	0	1
	<i>espJ</i>	0	3	0	2	0	1
	<i>espP</i>	13	8	13	16	4	13
	<i>nleA, nleB, nleC</i>	0	3	0	3	0	1
	<i>tir</i>	0	3	0	2	0	1
	<i>cif</i>	0	3	0	2	0	1
	<i>tccP</i>	0	2	0	1	0	1
	Toxins	<i>astA</i>	9 <sup>c</sup>	2	1	6	2
<i>stx1</i>		0	2	0	2	0	0
<i>stx1</i>		0	1	0	1	0	0
<i>stx2</i>		0	0	0	0	0	0
<i>α-hlyA</i>		0	3	0	2	0	1
Hemolysin	<i>ipfA</i>	9	8	7	11	3	10
	<i>prfB/papB</i>	5	2	3	3	1	6
	<i>iha</i>	7	5	6	8	2	8
	F17 (A, G)	2	0	0	1	0	1
	<i>fim41</i>	0	2	0	2	0	0
	<i>fanA</i>	0	2	0	2	0	0
	H <sup>d</sup>	1	6	4	8	0	2
	<i>saa</i>	0	1	3	4	0	0
	<i>efa1</i>	0	3	0	2	0	1
	<i>toxB</i>	0	2	0	1	0	1
Bacteriocins	<i>cma</i>	0	2	1	2	1	0
	<i>mchB, mchC</i>	6	3	7	7	3	5
Bacteriocin transporter	<i>mchH</i>	7	4	7	8	3	6
	<i>mcmA</i>	3	2	3	2	1	4
	<i>cba</i>	0	3	0	3	1	0
	<i>celB</i>	4	1	1	3	2	0
SPATE <sup>e</sup>	<i>espI</i>	0	1	0	1	0	0
	<i>pic</i>	0	0	1	1	0	0
Siderophore receptors	<i>ireA</i>	5	1	4	5	0	4
	<i>iroN</i>	0	1	1	1	0	0
Enzymes	<i>gad</i>	10	8	12	14	4	11
	<i>katP</i>	0	2	0	1	0	1

<sup>a</sup> A total of 41 of the 72 virulence genes and gene variants were detected in at least one ESBL-producing *E. coli* isolate by DNA microarray. The *bfpA*, *cdtB*, *cfaC*, *cnf*, *fasA*, *hlyE*, *ipaH*, *K88ab*, *IngA*, *IthA*, *perA*, *pet*, *senB*, *sfaS*, *sta1B*, *stb*, *stx2A*, *virF*, *aaaA* (*aaaC*), *eatA*, *epeA*, *espI*, *etpD*, *rpeA*, *sat*, *sepA*, *sigA*, *stxB2*, *subA*, *tsh*, and *vat* virulence factor genes were not detected. The percentages of recovery, calculated as (number of different VF genes detected × 100)/(number of virulence genes detected at least in one ESBL-producing *E. coli* strain), are 40.5% for phylogroup A, 90.5% for phylogroup B1, 45.2% for phylogroup D (global *P* value, <0.05 by the chi-square test), 92.9% for CTX-M-1, 33.3% for CTX-M-2, and 64.3% for CTX-M-9 (global *P* value, <0.05 by the chi-square test). The mean numbers of VF genes detected per isolate are 7.5 for phylogroup A, 10.8 for phylogroup B1 (*P*, <0.05 by ANOVA), 6.8 for phylogroup D, 8.2 for CTX-M-1, 8.3 for CTX-M-2, and 8 for CTX-M-9.

<sup>b</sup> The only *bla*<sub>TEM</sub> ESBL was excluded.

<sup>c</sup> Global *P* value, <0.05 by the chi-square test.

<sup>d</sup> H flagellar antigen genes were detected by real-time PCR (28).

<sup>e</sup> SPATE, serine protease autotransporters of *Enterobacteriaceae*.

ticularly in feces from diarrheic calves (182/204 [89.2%]), for which expanded-spectrum and “fourth-generation” cephalosporins are not the first-line treatment. However, most ESBL carriers proved to be multiresistant, suggesting that the use of antimicrobials other than beta-lactams, such as sulfonamides or coamoxiclav, may now contribute to the selection of resistance to expanded-spectrum and fourth-generation cephalosporins in this animal reservoir. A significant proportion (16.4%) of ESBLs of the CTX-M-1 group were CTX-M-15 enzymes, and this appears as a recur-

rent feature in this animal species (19). However, we could not link the types of ESBLs with the geographic origin of the specimens or any other epidemiological data.

Surprisingly, the ESBL-producing *E. coli* isolate of the O111:H8 serotype remained the only one to carry both the *stx* and *eae* genes. Two other isolates also carried the *eae* gene but did not possess the *stx* gene, and only one of these two isolates was characterized as belonging to a major EHEC serotype (O26:H11). Taken together, these findings indicate that the proportion of *E.*

*coli* ESBL carriers from cattle that are considered potential EHEC strains is quite low. This was rather unexpected, since ESBLs have been abundantly reported in cattle (19, 23, 26), and cattle have been recognized as the main EHEC animal reservoir (9). This is, however, in accordance with the very limited number of reports of ESBL-producing EHEC strains in humans (7, 17, 20). In cattle, however, the reasons why the subpopulation of EHEC isolates is still being spared from the widespread movement of the *bla*<sub>CTX-M</sub> genes within the *E. coli* population remain to be established.

We also showed that *E. coli* clinical isolates from cattle carrying ESBLs, collected over 5 years, belonged mainly to phylogroup A and, to a lesser extent, to phylogroups D and B1, whereas B2 strains were quasi-absent (1/204). In domestic animals, the underrepresentation of B2 strains had been already reported, but B1 is usually considered the main phylogroup (43, 45). However, none of the data published so far were gathered from a collection of ESBL-producing *E. coli* isolates, suggesting that antibiotic treatment may have possibly contributed to a different distribution of the phylogenetic groups.

On the other hand, in our collection, virulence factors were clearly associated with phylogroup B1, to a greater extent than with phylogroup A or D. Similar conclusions had been reported by a group from South Korea, which showed that the greatest number of strains carrying virulence genes were phylogroup B1 strains from beef and dairy cattle (46), although these were not from a collection of ESBL isolates. This suggests that the movement of the ESBL genes does not interfere significantly with the distribution of virulence factors within a particular phylogroup.

In this collection, even though the intimin-encoding gene *eae* was poorly represented and the virulent B1 phylogroup accounted for only 15.6% of the isolates, some virulence factors were found in isolates of phylogroups A and D. Considering that those isolates were also recovered from diarrheic specimens, this finding indicates that the panel of combinations of genes involved in intractable pathogenicity in cattle still remains to be fully understood. It could also be argued that some of these isolates were possibly not the cause of diarrhea. If this were true, it would suggest that ESBL genes are disseminated more efficiently within commensal than pathogenic *E. coli* isolates, whether they are pathogenic to calves or to humans.

The present study is a first contribution to the assessment of the pathogenicity and genetic background of ESBL-producing *E. coli* isolates from cattle. In contrast to previous reports for humans (6), we could not evidence any association between certain ESBL subgroups and specific virulence phenotypes or phylogroups (39). Although the underrepresentation of certain ESBL groups and virulence factors in this study is a limiting factor to this statement, the results of this study argue for coincidental combinations of virulence and resistance traits within *E. coli* isolates in cattle, which should result from local adaptive strategies of the *E. coli* population facing variable environments, in particular different antibiotic exposures. In certain cases, the ability of the *E. coli* species to harbor new combinations of genes may lead to highly aggressive strains from animals or the environment, as in the recent *E. coli* O104:H4 outbreak (5, 30). On the other hand, in contrast to human isolates, little is known about the genetic structure of the *E. coli* population in each animal species. Indeed, a follow-up study focused on ESBL-producing *E. coli* isolates from healthy cattle, to further compare how ESBL genes and virulence factors are distributed among different phylogroups and commensal or pathogenic

backgrounds of *E. coli* in the absence of antibiotic exposure, would be of great interest. The distribution of virulence genes would also be valuably compared with the genetic backgrounds among non-ESBL-producing *E. coli* isolates.

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## REFERENCES

1. Afset JE, et al. 2006. Identification of virulence genes linked with diarrhea due to atypical enteropathogenic *Escherichia coli* by DNA microarray analysis and PCR. *J. Clin. Microbiol.* 44:3703–3711.
2. Anjum MF, et al. 2007. Pathotyping *Escherichia coli* by using miniaturized DNA microarrays. *Appl. Environ. Microbiol.* 73:5692–5697.
3. Reference deleted.
4. Bielaszewska M, et al. 2011. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect. Dis.* 11:671–676.
5. Bielaszewska M, Sonntag AK, Schmidt MA, Karch H. 2007. Presence of virulence and fitness gene modules of enterohemorrhagic *Escherichia coli* in atypical enteropathogenic *Escherichia coli* O26. *Microbes Infect.* 9:891–897.
6. Branger C, et al. 2005. Genetic background of *Escherichia coli* and extended-spectrum beta-lactamase type. *Emerg. Infect. Dis.* 11:54–61.
7. Buvens G, Bogaerts P, Glupczynski Y, Lauwers S, Pierard D. 2010. Antimicrobial resistance testing of verocytotoxin-producing *Escherichia coli* and first description of TEM-52 extended-spectrum beta-lactamase in serogroup O26. *Antimicrob. Agents Chemother.* 54:4907–4909.
8. Canton R, Coque TM. 2006. The CTX-M beta-lactamase pandemic. *Curr. Opin. Microbiol.* 9:466–475.
9. Caprioli A, Morabito S, Brugere H, Oswald E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.* 36:289–311.
10. Cebula TA, Payne WL, Feng P. 1995. Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. *J. Clin. Microbiol.* 33:248–250.
11. Clermont O, et al. 2011. Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. *Infect. Genet. Evol.* 11:654–662.
12. CLSI. 2011. Performance standards for antimicrobial susceptibility testing, 21st international supplement. CLSI M100–S21. Clinical and Laboratory Standards Institute, Wayne, PA.
13. Cookson AL, Bennett J, Nicol C, Thomson-Carter F, Attwood GT. 2009. Molecular subtyping and distribution of the serine protease from Shiga toxin-producing *Escherichia coli* among atypical enteropathogenic *E. coli* strains. *Appl. Environ. Microbiol.* 75:2246–2249.
14. Coque TM, Baquero F, Canton R. 2008. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill.* 13:pii=19044. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19044>.
15. EFSA. 2009. Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food. *EFSA J.* 7:1366–1409.
16. Ibekwe AM, Grieve CM. 2003. Detection and quantification of *Escherichia coli* O157:H7 in environmental samples by real-time PCR. *J. Appl. Microbiol.* 94:421–431.
17. Ishii Y, et al. 2005. Extended-spectrum beta-lactamase-producing Shiga toxin gene (*stx*)<sub>1</sub>-positive *Escherichia coli* O26:H11: a new concern. *J. Clin. Microbiol.* 43:1072–1075.
18. Kim SH, Jia W, Bishop RE, Gyles C. 2004. An *msbB* homologue carried in plasmid pO157 encodes an acyltransferase involved in lipid A biosynthesis in *Escherichia coli* O157:H7. *Infect. Immun.* 72:1174–1180.
19. Kirchner M, Wearing H, Hopkins KL, Teale C. 2011. Characterization of plasmids encoding cefotaximases group 1 enzymes in *Escherichia coli* recovered from cattle in England and Wales. *Microb. Drug Resist.* 17:463–470.
20. Kon M, et al. 2005. Cefotaxime-resistant Shiga toxin-producing *Esche-*

- richia coli* O26:H11 isolated from a patient with diarrhea. *Kansenshogaku Zasshi* 79:161–168. (In Japanese.)
21. Lee K, Yong D, Yum JH, Kim HH, Chong Y. 2003. Diversity of TEM-52 extended-spectrum beta-lactamase-producing non-typhoidal *Salmonella* isolates in Korea. *J. Antimicrob. Chemother.* 52:493–496.
  22. Leyton DL, Sloan J, Hill RE, Doughty S, Hartland EL. 2003. Transfer region of pO113 from enterohemorrhagic *Escherichia coli*: similarity with R64 and identification of a novel plasmid-encoded autotransporter, EpeA. *Infect. Immun.* 71:6307–6319.
  23. Liebana E, et al. 2006. Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. *J. Clin. Microbiol.* 44:1630–1634.
  24. Literacka E, et al. 2009. *bla*<sub>CTX-M</sub> genes in *Escherichia coli* strains from Croatian hospitals are located in new (*bla*<sub>CTX-M-3a</sub>) and widely spread (*bla*<sub>CTX-M-3a</sub> and *bla*<sub>CTX-M-15</sub>) genetic structures. *Antimicrob. Agents Chemother.* 53:1630–1635.
  25. Livermore DM, et al. 2007. CTX-M: changing the face of ESBLs in Europe. *J. Antimicrob. Chemother.* 59:165–174.
  26. Madec JY, et al. 2008. Prevalence of fecal carriage of acquired expanded-spectrum cephalosporin resistance in *Enterobacteriaceae* strains from cattle in France. *J. Clin. Microbiol.* 46:1566–1567.
  27. Madec JY, et al. 2012. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*<sub>CTX-M-15</sub>-carrying plasmids. *J. Antimicrob. Chemother.* 67:578–581.
  28. Madic J. 2010. Détection et caractérisation moléculaire des principaux sérogroupes d'*Escherichia coli* producteurs de Shiga-toxines (STEC) associés aux Syndromes Hémolytiques et Urémique (SHU) et aux Colites Hé-morragiques (CH). Application à l'évaluation du risque STEC dans les aliments. Ph.D. dissertation. Ecole doctorale ABIES, Paris, France.
  29. Madic J, et al. 2011. Detection of Shiga toxin-producing *Escherichia coli* serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR. *Appl. Environ. Microbiol.* 77:2035–2041.
  30. Mellmann A, et al. 2011. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. *PLoS One* 6(7):e22751. doi: 10.1371/journal.pone.0022751.
  31. Meunier D, et al. 2010. Plasmid-borne florfenicol and ceftiofur resistance encoded by the *floR* and *bla*<sub>CMY-2</sub> genes in *Escherichia coli* isolates from diseased cattle in France. *J. Med. Microbiol.* 59:467–471.
  32. Meunier D, Jouy E, Lazizzera C, Kobisch M, Madec JY. 2006. CTX-M-1- and CTX-M-15-type beta-lactamases in clinical *Escherichia coli* isolates recovered from food-producing animals in France. *Int. J. Antimicrob. Agents* 28:402–407.
  33. Monday SR, Beisaw A, Feng PC. 2007. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. *Mol. Cell. Probes* 21:308–311.
  34. Naseer U, Sundsfjord A. 2011. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microb. Drug Resist.* 17:83–97.
  35. Nicolas-Chanoine MH, et al. 2008. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J. Antimicrob. Chemother.* 61:273–281.
  36. Paiva de Sousa C, Dubreuil JD. 2001. Distribution and expression of the *astA* gene (EAST1 toxin) in *Escherichia coli* and *Salmonella*. *Int. J. Med. Microbiol.* 291:15–20.
  37. Paton AW, Paton JC. 1999. Direct detection of Shiga toxigenic *Escherichia coli* strains belonging to serogroups O111, O157, and O113 by multiplex PCR. *J. Clin. Microbiol.* 37:3362–3365.
  38. Peirano G, Pitout JD. 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.
  39. Pitout JD, Laupland KB, Church DL, Menard ML, Johnson JR. 2005. Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum beta-lactamases. *Antimicrob. Agents Chemother.* 49:4667–4670.
  40. Roest HIJ, et al. 2007. Antibiotic resistance in *Escherichia coli* O157 isolated between 1998 and 2003 in The Netherlands. *Tijdschr. Diergeneesk.* 132:954–958. (In Dutch.)
  41. Rogers BA, Sidjabat HE, Paterson DL. 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J. Antimicrob. Chemother.* 66:1–14.
  42. Shibata N, et al. 2006. PCR classification of CTX-M-type beta-lactamase genes identified in clinically isolated gram-negative bacilli in Japan. *Antimicrob. Agents Chemother.* 50:791–795.
  43. Son I, Van Kessel JA, Karns JS. 2009. Genotypic diversity of *Escherichia coli* in a dairy farm. *Foodborne Pathog. Dis.* 6:837–847.
  44. Szmolka A, Anjum MF, La Ragione RM, Kaszanyitzky EJ, Nagy B. 2012. Microarray based comparative genotyping of gentamicin resistant *Escherichia coli* strains from food animals and humans. *Vet. Microbiol.* 156:110–118.
  45. Tenailon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of commensal *Escherichia coli*. *Nat. Rev. Microbiol.* 8:207–217.
  46. Unno T, et al. 2009. Absence of *Escherichia coli* phylogenetic group B2 strains in humans and domesticated animals from Jeonnam Province, Republic of Korea. *Appl. Environ. Microbiol.* 75:5659–5666.
  47. Valat C, et al. 2012. CTX-M-15 extended-spectrum beta-lactamase in a Shiga toxin-producing *Escherichia coli* isolate of serotype O111:H8. *Appl. Environ. Microbiol.* 78:1308–1309.