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Molecular Epidemiology of Dairy Cattle-Associated *Escherichia coli* Carrying bla_{CTX-M} Genes in Washington State

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ABSTRACT An increase in the prevalence of commensal *Escherichia coli* carrying bla_{CTX-M} genes among dairy cattle was observed between 2008 and 2012 in Washington State. To study the molecular epidemiology of this change, we selected 126 bla_{CTX-M}-positive and 126 bla_{CTX-M}-negative isolates for determinations of the multilocus sequence types (MLSTs) and antibiotic resistance phenotypes from *E. coli* obtained during a previous study. For 99 isolates, we also determined the bla_{CTX-M} alleles using PCR and sequencing and identified the replicon types of bla_{CTX-M}-carrying plasmids. The bla_{CTX-M}-negative *E. coli* isolates comprised 76 sequence types (STs) compared with 32 STs in bla_{CTX-M}-positive *E. coli* isolates. The bla_{CTX-M}-positive *E. coli* isolates formed three MLST clonal complexes, accounting for 83% of these isolates; 52% of bla_{CTX-M}-negative *E. coli* isolates clustered into 10 clonal complexes, and the remainder were singletons. Overall, bla_{CTX-M}-negative *E. coli* isolates had more diverse genotypes that were distinct to farms, whereas bla_{CTX-M}-positive *E. coli* isolates had a clonal population structure and were widely disseminated on farms in both regions included in the study. Plasmid replicon types included IncI1 which predominated, followed by IncFIB and IncFIA/FIB. $bla_{CTX-M-15}$ was the predominant CTX-M gene allele, followed by $bla_{CTX-M-27}$ and $bla_{CTX-M-14}$. There was no significant association between plasmid replicon types and bacterial STs, and neither clonal complexes nor major plasmid groups were associated with two discrete dairy-farming regions of Washington State.

IMPORTANCE Infections caused by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* occur globally and present treatment challenges because of their resistance to multiple antimicrobial drugs. Cattle are potential reservoirs of ESBL-producing *Enterobacteriaceae*, and so understanding the causes of successful dissemination of bla_{CTX-M} genes in commensal bacteria will inform future approaches for the prevention of antibiotic-resistant pathogen emergence.

KEYWORDS *Escherichia coli*, extended-spectrum β-lactamases, dairy cattle, molecular epidemiology, plasmids, sequence types

Infections caused by *Enterobacteriaceae* such as *Escherichia coli* or *Klebsiella pneumoniae* that are resistant to third-generation cephalosporins are a public health concern because they are challenging to treat, and their prevalence has increased in the United States (1) and worldwide (2) in recent decades. *Enterobacteriaceae* exhibit resistance to expanded-spectrum cephalosporins mainly by producing extended-spectrum β-lactamases (ESBLs), which are enzymes that hydrolyze β-lactam antibiotics. The ESBL families include TEM, SHV, OXA, CTX-M, and other variants. The CTX-M β-lactamases are of particular interest because they have become the predominant ESBL worldwide (3–5), and more than 120 CTX-M enzymes have been described (6, 7). The success of CTX-M β-lactamase-producing lineages has been attributed to several

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factors, such as an efficient mobilization and dissemination of CTX-M genes by mobile genetic elements (insertion sequences, transposons, and plasmids), the association of plasmids that harbor CTX-M genes with successful bacterial clones that are widely disseminated, the low fitness cost of CTX-M β-lactamase production, and antibiotic selection pressure (7).

The global problem of *E. coli* isolates that are resistant to critically important antibiotics requires investigations of how and where antibiotic selection is occurring, where reservoirs of resistant bacterial strains exist, and how these strains disseminate. We recently reported that *E. coli* isolated from dairy cattle in Washington State in 2008 did not carry CTX-M genes, while isolates collected in 2011 did carry CTX-M genes (8), confirming the recent emergence of these strains in the cattle population in that region. To investigate whether this relatively sudden widespread prevalence of $bla_{\text{CTX-M}}$-carrying *E. coli* isolates was due to the dissemination of one or a few clones, we applied multilocus sequence typing to determine the genotypes among $bla_{\text{CTX-M}}$-carrying and non-$bla_{\text{CTX-M}}$-carrying *E. coli* isolates and examined their distributions across farms and regions. To assess the possibility of plasmid horizontal transfer rather than clonal expansion, we also characterized CTX-M genes and plasmids that encode CTX-M enzymes and determined the associations between $bla_{\text{CTX-M}}$ alleles, plasmid replicon types, and genotypes. We also compared $bla_{\text{CTX-M}}$ alleles, plasmid replicon types, and genotypes from this study with those from other studies, particularly studies of $bla_{\text{CTX-M}}$-carrying *E. coli* isolates that are known to cause clinical infections in humans.

RESULTS

Comparison of MLST diversity and distribution between $bla_{\text{CTX-M}}$-positive and -negative *E. coli* isolates. The sequence type (ST) distribution was less diverse in *E. coli* isolates carrying $bla_{\text{CTX-M}}$ (Simpson's index of diversity [SID] = 0.98; 95% confidence interval [CI], 0.97 to 0.99) than in *E. coli* isolates lacking $bla_{\text{CTX-M}}$ (SID = 0.93; 95% CI, 0.91 to 0.95). We found 76 different STs among the 126 $bla_{\text{CTX-M}}$-negative *E. coli* isolates and 32 STs among the 126 $bla_{\text{CTX-M}}$-positive *E. coli* isolates. An eBURST analysis of the $bla_{\text{CTX-M}}$-positive *E. coli* isolates based on multilocus sequence type (MLST) genotypes identified three clonal complexes (CC10, CC58, and CC88), which accounted for 105 isolates (83.3% of the $bla_{\text{CTX-M}}$-positive *E. coli* isolates) and 21 singletons (see Table S3 in the supplemental material). CC10 was the predominant clonal complex, which included 6 STs and accounted for 50% of the $bla_{\text{CTX-M}}$-positive isolates, and was found on 16 of 18 farms sampled. Among the 63 $bla_{\text{CTX-M}}$-positive isolates within the CC10 complex, ST744 was most frequent (n = 20), followed by ST48 (n = 16) and ST761 (n = 15). CC58 was the second largest clonal complex, accounting for 9.5% of $bla_{\text{CTX-M}}$-positive *E. coli* isolates from 5 different farms. The third largest clonal complex (CC88) was also found on 5 separate farms. Overall, 65.1% of $bla_{\text{CTX-M}}$-positive *E. coli* isolates were associated with these three clonal complexes (Fig. 1; Table 1). As was true for $bla_{\text{CTX-M}}$-positive *E. coli* isolates, CC10 and CC58 were the most common clonal complexes among the $bla_{\text{CTX-M}}$-negative *E. coli* isolates but represented smaller proportions of the total (15.1% and 12.7%, respectively). The $bla_{\text{CTX-M}}$-negative *E. coli* isolates included a significantly higher proportion of singleton STs than the $bla_{\text{CTX-M}}$-positive *E. coli* isolates ($P < 0.05$) (Fig. 2; Table 1). When all of the isolates were analyzed together, the $bla_{\text{CTX-M}}$-negative and -positive *E. coli* isolates were often associated with the same or closely linked STs, with the exception of a few STs; for example, ST44, ST162, and ST361 had 4 to 6 isolates each and were exclusively $bla_{\text{CTX-M}}$ positive. The association between ST744, ST761, and ST48 and $bla_{\text{CTX-M}}$ carriage is noticeable in this analysis (Fig. 3).

Human disease-associated sequence types. ST69 (n = 4) and ST95 (n = 1) were detected among the $bla_{\text{CTX-M}}$-negative *E. coli* isolates, and one ST69 isolate was $bla_{\text{CTX-M}}$ positive. These sequence types are considered pandemic human disease-associated STs (Table 2; Fig. 1 and 2) (9).

Plasmid replicon types and CTX-M alleles among $bla_{\text{CTX-M}}$-positive *E. coli* isolates. Plasmid replicon typing was performed on 99 $bla_{\text{CTX-M}}$-positive *E. coli* isolates

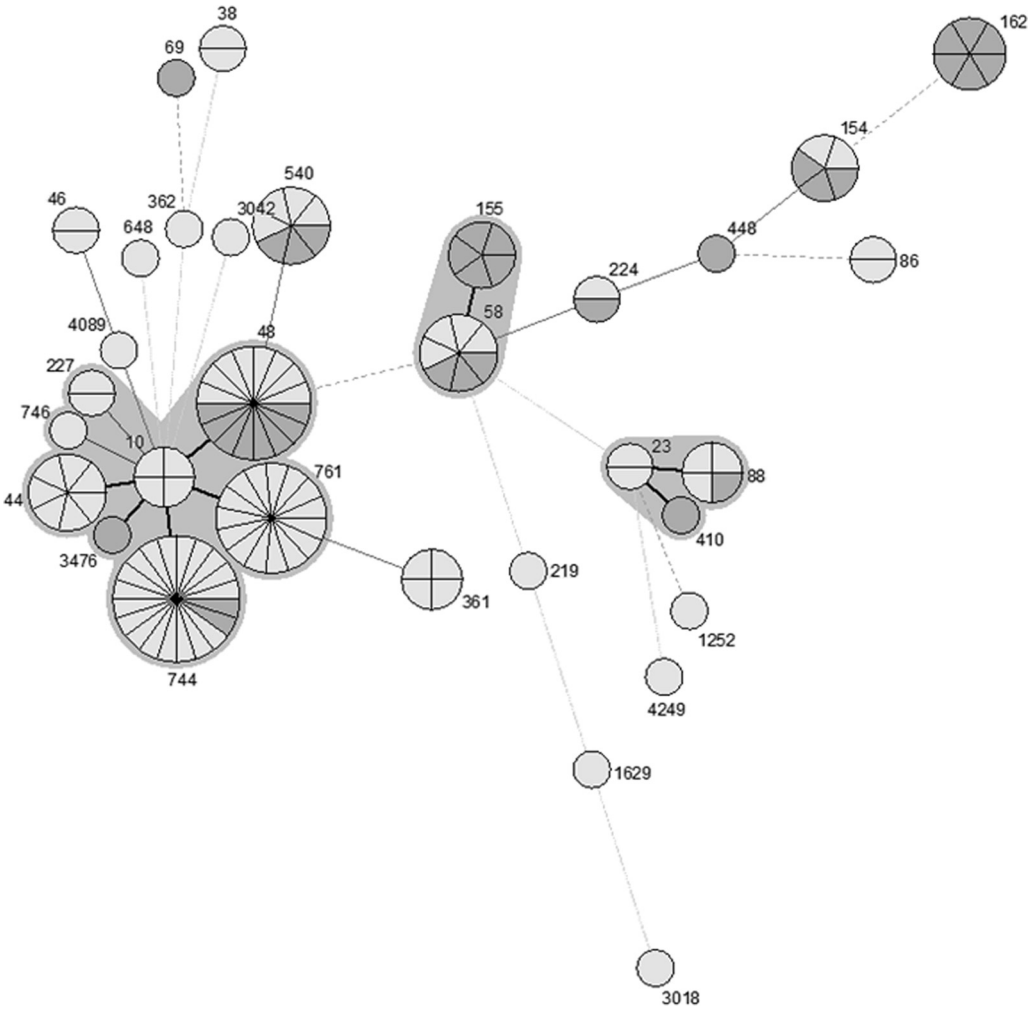

FIG 1 Minimum spanning tree analysis of 126 bla_{CTX-M}-positive *Escherichia coli* isolates based on MLST genotypes. Each circle represents a genotype, and the partitions within a circle are individual isolates. There are three main clonal complexes, and each is surrounded by a gray zone. Thick lines connect genotypes that differ at one locus, and thin lines connect genotypes that differ by two or more loci. Shading indicates the region from which isolates were obtained: pale gray indicates eastern Washington ($n = 78$) and dark gray indicates northwestern Washington ($n = 48$).

from which we were able to isolate the bla_{CTX-M}-carrying plasmids. Nine replicon types were identified among 89 isolates; an additional 10 isolates could not be typed using the multiplex PCR method (10, 11). IncI1 plasmids were most frequent ($n = 31$), followed by IncFIB ($n = 27$), IncFIA/FIB, IncN, IncFIA, and others (Table 3).

TABLE 1 Multilocus sequence types and clonal complexes observed in 126 bla_{CTX-M}-positive and 126 bla_{CTX-M}-negative *E. coli* isolates from cattle on 18 dairy farms in Washington State

	bla_{CTX-M}-positive *E. coli*		bla_{CTX-M}-negative *E. coli*	
Clonal complex[a]	ST	No. of isolates (%)	ST	No. of isolates (%)
10	10, 44, 48, 744, 761, 3476	63 (50)	10, 34, 48, 744, 761, 1137, 1415	19 (15.1)
58	58, 155	12 (9.5)	58, 155, 4091, 4092	16 (12.7)
88	23, 88, 90	7 (5.5)	23, 88, 90	5 (3.97)
29			21, 29, 16, 723, 765	11 (8.7)
No founder				14 (11.1)
Singletons		44 (34.9)		61 (48.4)
Total		126 (100)		126 (100)

[a]Clonal complexes were defined using the eBURST algorithm from bla_{CTX-M}-negative *E. coli* and bla_{CTX-M}-positive *E. coli* MLST data. The clonal complexes correspond to the predicted founder. "No founder" refers to the inability of the algorithm to predict the founder. The likelihood ratio chi-square *P* value was ≤0.001 for a 2 × k test for differences in the percentages of each clonal complex within bla_{CTX-M}-negative and bla_{CTX-M}-positive *E. coli* isolates.

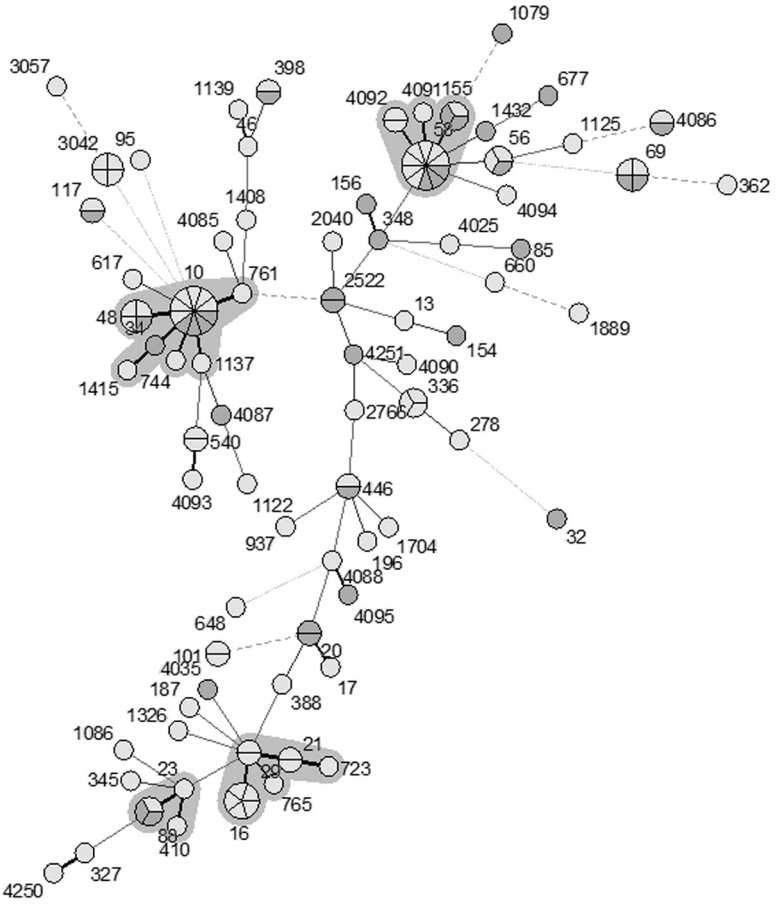

FIG 2 Minimum spanning tree analysis of 126 bla_{CTX-M}-negative *Escherichia coli* isolates based on MLST genotypes. Each circle or node represents a genotype, and the partitions within a node are individual isolates. Each clonal complex is surrounded by a gray zone. Thick lines connect genotypes that differ by one locus, and thin lines connect genotypes that differ by two or more loci. Shading indicates the region from which isolates were obtained: pale gray indicates eastern Washington ($n = 78$) and dark gray indicates northwestern Washington ($n = 48$).

We detected 6 different bla_{CTX-M} alleles, of which the most frequent was $bla_{CTX-M-15}$ ($n = 50$), followed by $bla_{CTX-M-27}$ ($n = 26$) and $bla_{CTX-M-14}$ ($n = 16$), while $bla_{CTX-M-65}$, $bla_{CTX-M-24}$, and $bla_{CTX-M-55}$ were uncommon (Table 3). Overall, $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$ were widely distributed among the different STs. There was an association between MLST clonal complexes and CTX-M alleles ($P = 0.0008$); the isolates in CC10 tended to carry $bla_{CTX-M-15}$ or $bla_{CTX-M-27}$ alleles; those in CC88 mostly carried $bla_{CTX-M-15}$ alleles (Fig. 4).

There was an association between replicon types and bla_{CTX-M} alleles ($P = 0.007$). For instance, IncI1 and IncFIA/FIB predominantly carried $bla_{CTX-M-15}$ alleles, while IncFIB carried $bla_{CTX-M-27}$, $bla_{CTX-M-15}$, and $bla_{CTX-M-14}$ alleles in almost equal proportions. There was no clear association between clonal complex and plasmid replicon type, including the 10 plasmids that were nontypeable ($P = 0.178$) (Table 2).

Geographic distribution of MLSTs. The four major clonal complexes did not differ significantly in the proportions that were found in the two regions of Washington State (Table S3).

Antibiotic resistance among bla_{CTX-M}-positive and bla_{CTX-M}-negative *E. coli* isolates. The proportions of bla_{CTX-M}-positive *E. coli* isolates with resistance to ampicillin, ceftiofur, cephalothin, chloramphenicol, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole were higher than those of bla_{CTX-M}-negative isolates (Table 4).

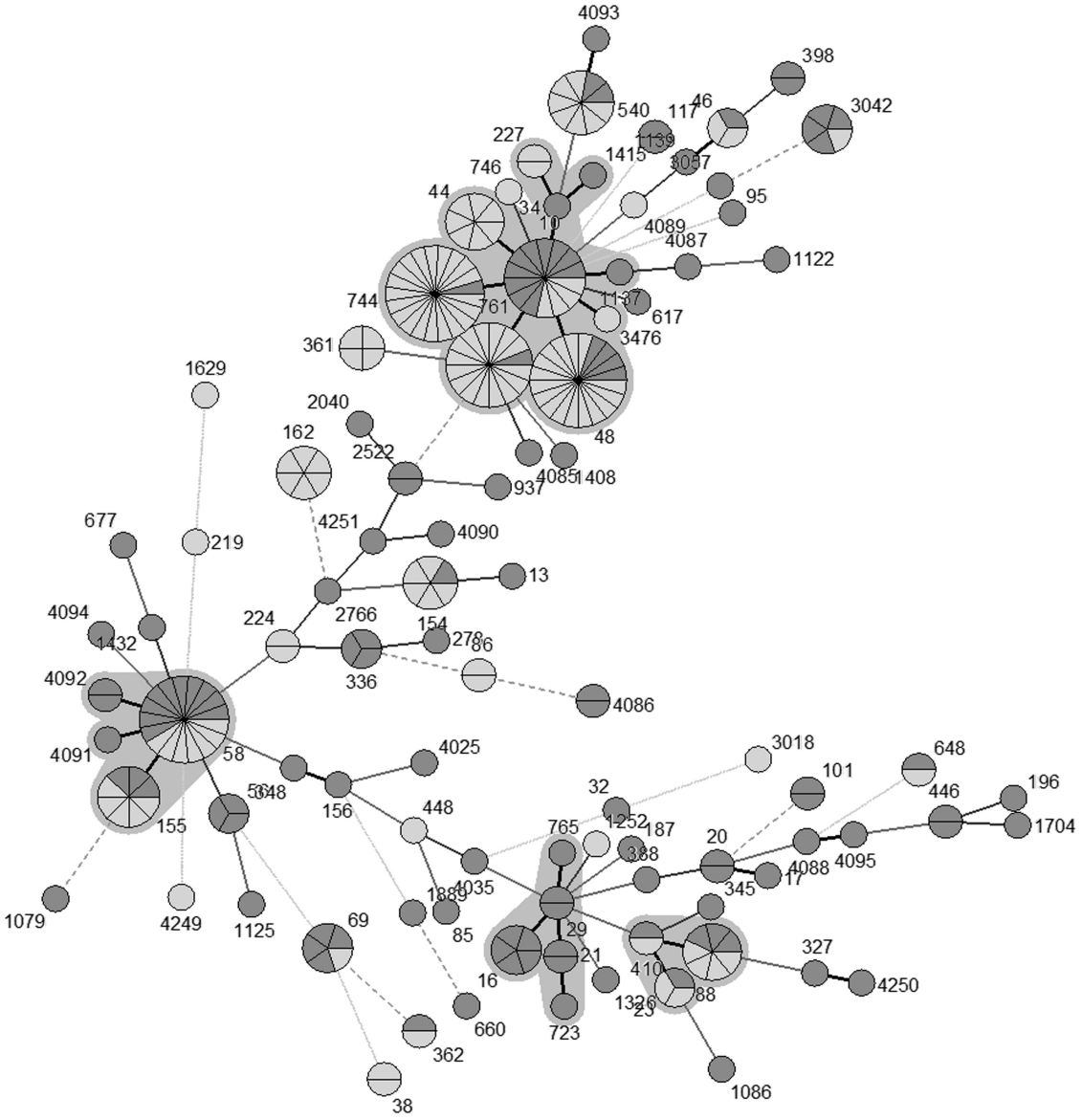

FIG 3 Minimum spanning tree analysis of 252 *Escherichia coli* isolates based on MLST genotypes. Each circle represents a sequence type, and the partitions within each circle represent individual isolates. Clonal complexes are surrounded by gray zones. Within each circle, dark gray represents bla_{CTX-M}-positive *Escherichia coli* isolates ($n = 126$) and light gray represents bla_{CTX-M}-negative *Escherichia coli* isolates ($n = 126$).

DISCUSSION

We recently reported an increase in commensal *E. coli* isolates carrying bla_{CTX-M} resistance genes in dairy cattle, and to assess the possibility that this represented the clonal dissemination of one or a few clones of *E. coli*, we genotyped bla_{CTX-M}-positive and bla_{CTX-M}-negative commensal *E. coli* isolates from dairy cattle across Washington State. We found that a large proportion of bla_{CTX-M}-positive *E. coli* isolates belonged to a few MLST clonal complexes, while bla_{CTX-M}-negative *E. coli* isolates were significantly more diverse with regard to sequence types. The predominance of a few clones among bla_{CTX-M}-positive *E. coli* isolates is consistent with other studies that report few genotypes accounting for a large proportion of multidrug-resistant strains (9).

The primary clonal complexes of commensal bla_{CTX-M}-positive *E. coli* isolates were a subset of those found in bla_{CTX-M}-negative *E. coli* isolates, suggesting a scenario in which the acquisition of bla_{CTX-M} plasmids by *E. coli* genotypes that were already fit and established in the dairy animal niche resulted in an increase in their fitness in the

TABLE 2 Distribution of CTX-M genes and plasmid replicon types among 99 bla_{CTX-M}-positive *E. coli* isolates from dairy cattle in Washington State

		No. of isolates																
		CTX-M group 1		CTX-M group 9				Plasmid replicon types										
CC[a]	ST[b]	CTX-M-15	CTX-M-55	CTX-M-14	CTX-M-24	CTX-M-27	CTX-M-65	I1	FIB	NT	FIA/FIB	N	FIA	F	FIA/I1	B/0	A/C	
CC10	ST761	4			1	8		3	5	1		1		2				
	ST48	1		1		5		2	2			1	3					
	ST744	10		2		4		7	3	2	1	2		1				
	ST10					2		1				1						
	ST4096					1			1									
	ST44	5						1			4							
	ST685						1		1									
CC58	ST58	4				2		4	2									
	ST155			5					4							1		
CC88	ST23					1				1								
	ST88	9						3	2	3	1							
	ST90	1												1				
Singletons or no founders	ST162			3					2		1							
	ST86	2		1				3										
	ST361	4							1	1	1				1			
	ST540			2			3	1		1		2	1					
	ST1163		1			1			1				1					
	ST154	1								1								
	ST345	1						1										
	ST448			1									1					
	ST219	1						1										
	ST1629					1		1										
	ST3018	1											1					
	ST32					1			1									
	ST4249	1							1									
	ST227	1						1										
	ST3042	1						1										
	ST163			1														
	ST362	1						1										
	ST69						1					1						
	ST746	1									1							
	ST38																1	
	ST349	1							1									
Total		50	1	16	1	26	5	31	27	10	9	8	7	4	1	1	1	

[a]CC, clonal complex.
[b]ST, sequence type.

presence of antimicrobial selection pressure. The wide distribution of bla_{CTX-M}-positive *E. coli* clones across a broad geography could be the result of clonal dissemination via movement of cattle, humans, contaminated vehicles, feed, and possibly birds and insects (8, 12, 13), followed by a diversification through normal microevolutionary mechanisms, such as mutation and horizontal gene transfer mediated by plasmids and bacteriophages (14, 15). An expectation of this scenario would be that a single ST of *E. coli* could be associated with a diversity of plasmid types, which is consistent with our observations. For example, in our data, ST744 isolates ($n = 16$) were associated with six different plasmid types associated with three different bla_{CTX-M} alleles (Table 2).

We detected small numbers of ST69 and ST95, which, like ST131 and ST393, have been referred to as pandemic clonal lineages of human-associated extraintestinal pathogenic *E. coli* (ExPEC). The ST69 isolates in this study were primarily bla_{CTX-M} negative, which is consistent with reports that ST69 strains rarely produce ESBL. To our knowledge, these were the only human disease-associated pandemic STs detected in our study (9). It has been suggested that food-producing animals are reservoirs of resistant bacteria that cause clinical infections in humans (16–18); however, compelling quantitative evidence is lacking. For example, a systematic review of 34 original studies

TABLE 3 Relationship of plasmid replicon types and CTX-M genes among 99 bla_{CTX-M}-positive *E. coli* isolates from cattle on dairy farms in Washington State

	No. of isolates						
	CTX-M group 1		CTX-M group 9				
Plasmid replicon type	CTX-M-15	CTX-M-55	CTX-M-14	CTX-M-24	CTX-M-27	CTX-M-65	Subtotal
IncI1	24		2		4	1	31
IncFIB	8		7	1	10	1	27
IncFIA/FIB	7		1		1		9
IncFIA	1	1	2		3		7
IncF	2				2		4
IncFIA/I1	1						1
IncA/C			1				1
IncB/O			1				1
IncN	1				4	3	8
NT[a]	6		2		2		10
Total	50	1	16	1	26	5	99

[a]NT, nontypeable: no PCR product was obtained using the standard set of primers for replicon typing (10).

found that six studies provided evidence for whole bacterium transmission, and 13 studies provided evidence for the transmission of resistance via mobile genetic elements from food animals to humans; most of these were of poultry or retail meats. Conversely, findings from 17 studies did not support whole bacterium transmission, and two studies did not provide evidence for the transfer of resistance via mobile genetic elements from food animal sources to humans (19).

Among the STs within the ST10 clonal complex, ST48, ST744, and ST761 were the

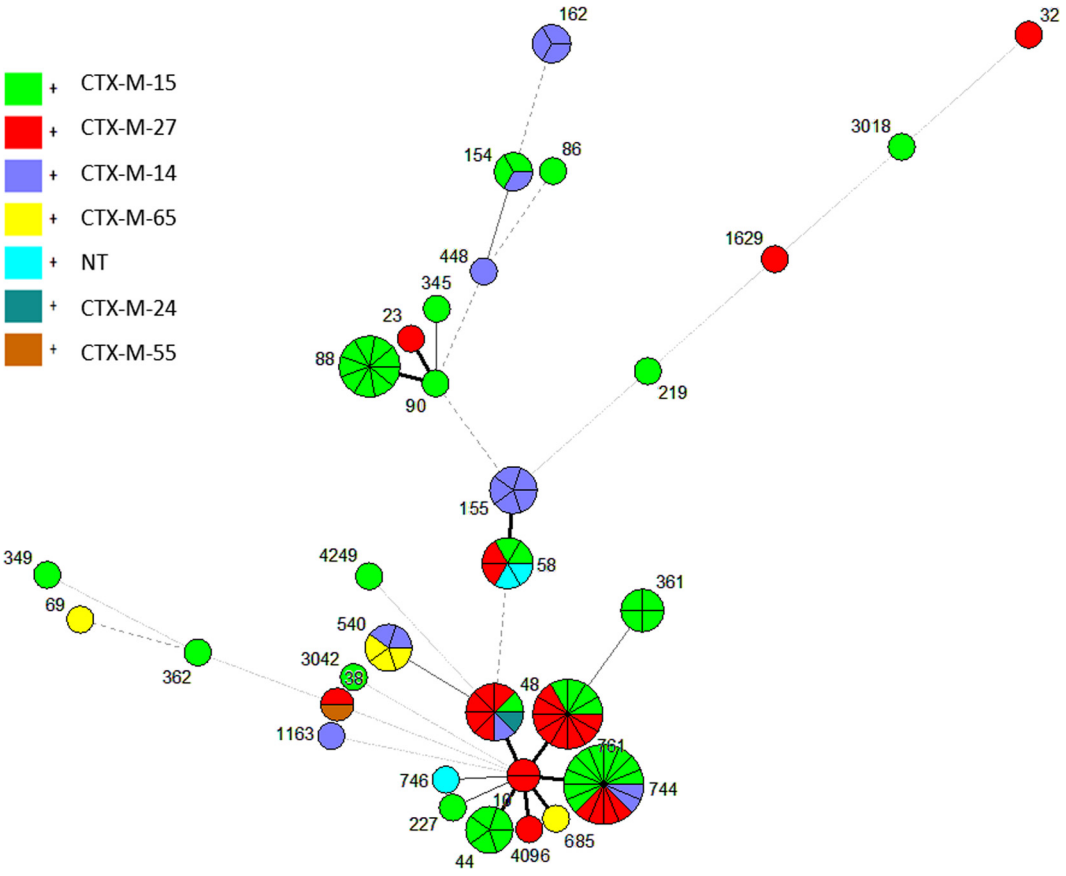

FIG 4 Distributions of bla_{CTX-M} genes among the different MLST genotypes in bla_{CTX-M}-positive *E. coli* isolates (n = 99) from dairy cattle in Washington State. NT, nontypeable.

TABLE 4 Percentages of bla_{CTX-M}-positive and bla_{CTX-M}-negative *E. coli* isolates resistant to a panel of 12 antibiotics

Antibiotic	Point estimate (% [95% CI])	
	CTX-M-positive *E. coli*	CTX-M-negative *E. coli*
Amikacin	4.0 (1.7–9.0)	4.7 (2.2–10.0)
Amoxicillin-clavulanic acid	15.1 (9.9–22.3)	22.2 (15.8–30.2)
Ampicillin	99.2 (95.6–100)[a]	38.9 (30.8–47.6)[a]
Ceftiofur	99.2 (95.6–100)[a]	13.4 (8.6–20.5)[a]
Cephalothin	99.2 (95.6–100)[a]	27.0 (20.0–35.3)[a]
Chloramphenicol	52.3 (43.7–60.9)[a]	30.2 (22.8–38.7)[a]
Gentamicin	7.9 (4.4–14.0)	9.5 (5.5–15.9)
Nalidixic acid	35.7 (27.9–44.4)[a]	7.9 (4.4–14.0)[a]
Streptomycin	77.8 (69.8–84.2)[a]	46.0 (37.6–54.7)[a]
Sulfisoxazole	73.0 (64.7–80.0)[a]	45.2 (36.8–53.9)[a]
Tetracycline	86.5 (79.5–91.4)[a]	51.6 (43.0–60.1)[a]
Trimethoprim-sulfamethoxazole	66.7 (58.1–74.3)[a]	27.8 (20.7–36.2)[a]

[a]Higher percentage of bla_{CTX-M}-positive isolates are resistant than bla_{CTX-M}-negative isolates.

most frequent and had the strongest association with bla_{CTX-M} (Fig. 1 through 3). Although ST761 was not detected in the northwestern region of Washington State, the other two in that complex (ST48 and ST744) were detected in both regions, suggesting that they represent widely disseminated types (Fig. 2). ST744 has been frequently reported from widely dispersed locations around the world, including from food animals in Australia (20), broiler chickens in Algeria (21), bovine mastitis in Germany (22), cattle in France (23), and hospitalized patients in Hong Kong (24), many with ESBL and/or carbapenem resistance characteristics. ST48 has also frequently been reported from diverse sources and locations and is reportedly associated with resistance (25–30).

The IncI1 and IncF plasmid families were the most frequent replicon types detected in this study, which is consistent with reports that IncI1 and IncF were the most frequent replicon types among $bla_{CTX-M-15}$-carrying plasmids in *Enterobacteriaceae* from human, livestock, and environmental sources in European studies (31–33). IncFIB has been reported to be the most common replicon type in commensal and pathogenic *E. coli* isolates originating from avian, human, and poultry meat (34), and it has been associated with virulence (35). Both IncI and IncF plasmid groups are considered to be easily transmissible narrow-host-range plasmids and clustered with each other in an analysis of their gene content in comparison with that of IncN, IncP, and IncW plasmids (36, 37). Unlike IncI1 and IncFIA/FIB that were mainly associated with $bla_{CTX-M-15}$, IncFIB, the second most common replicon type in this study, was associated with $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, and $bla_{CTX-M-27}$ genes (Table 3).

$bla_{CTX-M-15}$ was the most frequent bla_{CTX-M} allele in this study, followed by $bla_{CTX-M-27}$ and $bla_{CTX-M-14}$ (Table 3). Consistent with our findings, $bla_{CTX-M-15}$ was the dominant allele among cattle-derived ESBL-producing *E. coli* isolates in the United Kingdom and Lebanon (38, 39). In contrast with our results, the prevalence of $bla_{CTX-M-15}$-producing *E. coli* was reported to be low among ESBL-producing *E. coli* isolates from nonhuman sources (33, 40). A review of ESBL genes in *E. coli* isolates from human and nonhuman sources from Europe and Asia found that overall, $bla_{CTX-M-1}$ was the most frequent allele in cattle/pig-derived isolates (72%), followed by $bla_{CTX-M-14}$ (14%) and $bla_{CTX-M-15}$ (8%); however, regional differences in prevalence were observed (40). Other studies indicate that $bla_{CTX-M-14}$ is prevalent among cattle-derived ESBL-producing *E. coli* isolates (41, 42), while $bla_{CTX-M-27}$ is uncommon in ESBL-producing *E. coli* isolates from cattle/pigs (40). Whereas the real explanation for the observed differences in the distribution of bla_{CTX-M} alleles is unknown, plausible explanations include environmental or geographical factors, differences in β-lactamase activity against specific antimicrobial substrates, regional founder effects, the mobility of plasmids that mediate these genes, and other resistance and virulence determinants (43, 44).

Conclusion. We observed low MLST diversity among commensal *E. coli* isolates carrying bla_{CTX-M} in dairy cattle across Washington State and a lack of association

between geographic region and MLST clonal complexes, suggesting that clonal bacterial spread played a role in the rapid increase of bla_{CTX-M} prevalence that was observed between 2008 and 2012 (8). However, we did not detect any association between the bla_{CTX-M} plasmid replicon types and the ST or clonal complexes of their host bacteria. In addition, the bla_{CTX-M}-negative *E. coli* isolates, although more diverse, included the same clonal complexes that were prominent among the bla_{CTX-M}-positive *E. coli* isolates. Certain *E. coli* STs are apparently host associated, while bla_{CTX-M} plasmid replicon types are found in multiple vertebrate hosts and have been referred to as "epidemic plasmids," suggesting an important role of horizontal gene transfer in the epidemiology of bla_{CTX-M}-associated *E. coli* (45).

To elucidate all of the causal factors driving a population change in resistant bacteria, we would need a complex model of multiple interacting forces, including animal movements, environmental distribution systems such as water flow, local antimicrobial use, and selective pressure, and the fitness of bacteria due to plasmid and other genetic acquisitions. Ideally, such a model could simulate changes in any of these factors to predict changes in commensal as well as clinical bacterial populations that may impact public health.

MATERIALS AND METHODS

Sample collection. Samples for this study were collected during the summer and fall of 2012 as described in a previous study (8). The 30 dairy farms in that study were chosen based on their willingness to collaborate and their geographic dispersion across the state. For 21 of the 30 farms sampled in that study, susceptible *E. coli* isolates with no bla_{CTX-M} were available because nonselective as well as selective media were used, but three of those 21 farms had no *E. coli* isolates carrying bla_{CTX-M}. To be able to compare the genotypes of *E. coli* isolates with no bla_{CTX-M} to genotypes of *E. coli* isolates carrying bla_{CTX-M}, we used isolates from the remaining 18 dairy farms. They were located in northwestern (region 1) and eastern (region 2) Washington State. Fecal samples were collected from the rectums of individual calves or pooled from fresh fecal pats from adult lactating cow pens, were placed on ice, and were transported to the laboratory for processing (8).

Bacterial culture, isolation, and confirmation. *Escherichia coli* isolates were cultured from fecal samples as previously described (46). Briefly, each sample was directly plated onto unsupplemented MacConkey agar and incubated overnight, and three presumptive *E. coli* colonies were inoculated in a 96-well plate containing brain heart infusion (BHI) broth and incubated. Buffered glycerol was added to each well, and the plates were stored at −80°C. In addition, to select for ESBL strains, each fecal sample (5 g) was placed in nutrient broth supplemented with cefotaxime (2 μg/ml) (Research Products International Corp., Mount Prospect, IL, USA) and incubated overnight. Then, 100 μl nutrient broth was plated onto MacConkey agar plates supplemented with cefepime (4 μg/ml) (Apotex Corp., Weston, FL, USA). Similarly, three single colonies were picked from each plate, inoculated in 96-well plates, and stored. The isolates were confirmed as *E. coli* by PCR amplification of the *uidA* gene (47).

Detection of the bla_{CTX-M} gene and isolate selection. The presence of bla_{CTX-M} was determined by PCR (48). We grouped preweaned calves into age groups of 7 days each (1 to 7 days, 8 to 14 days, 14 to 21 days, 22 to 28 days, 35 to 42 days, 43 to 49 days, 50 to 56 days, and 57 to 63 days) and adults into one age group. From each group, one to four samples were chosen by generating a random number list using the Rand function in Excel version 3 (Microsoft, Redmond, WA), sorting by random number within the group, and choosing the first isolate in the list. Because we selected similar numbers of bla_{CTX-M}-positive and -negative isolates from each age group, we could not make inferences about the distribution of CTX-M by animal age. From each of the 18 farms, an average of 14 isolates (standard deviation [SD], 5.5), including one to four from adult cows and 1 to 4 from each age group of preweaned calves, were chosen for a final isolate set of 126 bla_{CTX-M}-negative and 126 bla_{CTX-M}-positive isolates (total, 252 isolates). Ninety-six isolates were from farms in northwestern Washington and 156 isolates were from farms in eastern Washington (see Table S1 in the supplemental material). To add to the sample size of isolates for the purpose of studying the distribution of plasmid types and bla_{CTX-M} alleles, an additional 23 isolates from farms not included in the current study, but from which plasmids could be isolated, were used. These additional isolates were obtained from nine different farms in eastern Washington (region 2) during the previous study (8), one to three isolates per farm. They were obtained during the same time period as the 252 isolates that were chosen for the MLST comparison between bla_{CTX-M}-carrying and noncarrying *E. coli* isolates. Although northwestern Washington (region 1) was not represented in this additional set, the aim was to get as complete a picture as possible of replicon types, and so we used these for that purpose only (8, 49) (see Table S2).

Nucleic acid extraction. Each isolate was streaked on a blood agar plate, and a single colony was inoculated into 3 ml BHI broth and incubated overnight at 37°C. Genomic DNA from each isolate was extracted using a Qiagen DNeasy blood and tissue kit according to the manufacturer's instructions (Qiagen Sciences, Inc., Germantown, MD) and stored at 4°C.

MLST determination. All isolates were genotyped according to the Achtman seven-locus MLST scheme. Briefly, internal fragments of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) were amplified using published primers and protocols (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/

TABLE 5 PCR Primers used in this study

Target	Primer name	Sequence (5′→3′)	Reference
bla_{CTX-M}	Pan CTX-M forward	TTT GCG ATG TGC AGT ACC AGT AA	48
	Pan CTX-M reverse	CGA TAT CGT TGG TGG TGC CAT A	
bla_{CTX-M} (group I)	CTXM1-F3 forward	GAC GAT GTC ACT GGC TGA GC	59
	CTXM1-R2 reverse	AGC CGC CGA CGC TAA TAC A	
bla_{CTX-M} (group II)	TOHO1-2F forward	GCG ACC TGG TTA ACT ACA ATC C	59
	TOHO1-1R reverse	CGG TAG TAT TGC CCT TAA GCC	
bla_{CTX-M} (group III)	CTXM8.WSAgroupIII.F forward	AGA CCT GAT TAA CTA CAA TCC CAT TA	51
	CTXM8.WSAgroupIII.R reverse	ACT TTC TGC CTT CTG CTC TGG C	
bla_{CTX-M} (group IV)	CTXM914F forward	GCT GGA GAA AAG CAG CGG AG	59
	CTXM914R reverse	GTA AGC TGA CGC AAC GTC TG	
bla_{CTX-M} sequencing primers	CTXM-1 upstream forward	ATG TTG TTG TTA ATT CGT CTC	46
	CTXM-1 upstream reverse	CGT TAT CGC TGT ACT GTA G	
	CTXM-2 downstream forward	TTA ACT ATA ATC CGA TTG CG	46
	CTXM-2 downstream reverse	TTT CTG CCT TAG GTT GAG	
bla_{CTX-M} (CTX-M-9 group)	M9 upper forward	ATG GTG ACA AAG AGA GTG CA	60
	M9 lower reverse	CCC TTC GGC GAT GAT TCT C	
*uid*A	UAL-754 forward	AAA ACG GCA AGA AAA AGC AG	47
	UAR-900 Reverse	ACG CGT GGT TAC AGT CTT GCG	

documents/primersColi_html). The amplified products were sequenced bidirectionally, the raw trace files were read, and the sequences were assembled into single contigs using BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Each unique allele was assigned a number, the allelic profile for each isolate was determined, and sequence types (STs) were assigned using BioNumerics (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). The sequences of novel alleles were submitted to the curator for new allele and ST assignment. The genotypic relationships among isolates were determined by generating minimum spanning trees using BioNumerics. Within a clonal complex, each ST has to match with at least one other ST at 6 loci. The eBURST algorithm was used to determine the founder ST of a clonal complex (http://eburst.mlst.net/).

Antibiotic resistance testing. The 252 *E. coli* isolates were tested for resistance to 12 antibiotics (amikacin, 30 μg; amoxicillin-clavulanic acid, 20/10 μg; ampicillin, 10 μg; ceftiofur, 30 μg; cephalothin, 30 μg; chloramphenicol, 30 μg; gentamicin, 10 μg; nalidixic acid, 30 μg; streptomycin, 10 μg; sulfisoxazole, 0.25 μg; tetracycline, 30 μg; and trimethoprim-sulfamethoxazole, 1.25/23.75 μg) using the disc diffusion assay according to the Clinical and Laboratory Standards Institute guidelines (50).

CTX-M groups and types. To determine bla_{CTX-M} groups, we first screened for bla_{CTX-M} group 1 using previously described protocols and primers, followed by PCR to detect bla_{CTX-M} group 9 (51). To determine bla_{CTX-M} types, we performed PCR using sequencing primers as previously described (46, 52). The primers used to determine bla_{CTX-M} groups and types are shown in Table 5. The PCR products were submitted to Functional Biosciences (Madison, WI, USA) for sequencing, and the specific bla_{CTX-M} alleles were identified using the NCBI Basic Local Alignment Search Tool (53).

Plasmid isolation, characterization, and replicon typing. To isolate and characterize bla_{CTX-M}-associated plasmids, two procedures were used. Conjugation experiments were performed by growing bla_{CTX-M}-positive *E. coli* and GeneHog recipient cells (Invitrogen) separately in LB broth at 37°C for 6 h. Then, bla_{CTX-M}-positive *E. coli* and GeneHog recipient cells were plated together on LB medium supplemented with rifampin (100 μg/ml) and cefepime (16 μg/ml) to select for recipient cells carrying the bla_{CTX-M} plasmid. Conjugants were screened by denaturing chromosomal DNA using alkaline sodium dodecyl sulfate, removing protein and cell debris by phenol-chloroform extraction (54), and confirming the presence of bla_{CTX-M} using PCR (48). If conjugation was not successful, plasmid DNA was extracted using a GeneJET plasmid Midiprep kit (Thermo Fisher Scientific, Waltham, MA, USA) or by using a published protocol for larger plasmids (55). The plasmid DNA was then electroporated into competent *E. coli* DH10B or GeneHog cells (Invitrogen) in 2-mm-path-length cuvettes using a GenePulser (Bio-Rad). Transformants were immediately placed into SOC medium (MP Biomedicals, Solon, OH, USA) (500 ml) and incubated for 1 h at 37°C with agitation (300 rpm). Transformants were then plated on LB agar plates supplemented with cefepime (16 μg/ml). The isolates were confirmed to have a single plasmid by preparing plasmid DNA as above (54), and the bla_{CTX-M} gene was confirmed using PCR (48).

Plasmid replicon typing was performed as described by Johnson and Nolan (10), using a modification of the PCR method described by Carattoli et al. (11). The procedure involves three multiplex PCR panels using previously described primer pairs, targets, control strains, and run conditions, and the approach can detect 18 replicon types.

Statistical analyses. The proportions of bla_{CTX-M}-carrying and non-bla_{CTX-M}-carrying *E. coli* isolates that were resistant to the antibiotics tested were compared using R package Hmisc. The associations between genotype and bla_{CTX-M} allele and between plasmid replicon type and bla_{CTX-M} allele were determined using Pearson's chi-square test of independence in R software. The comparisons between proportions were tested for significance using the Pearson's chi-square test in the software WinPepi (56). The Simpson's index of diversity was calculated according to the formula developed by Hunter and Gaston (57), and 95% confidence intervals surrounding the indices of diversity were calculated using the formula of Grundmann et al. (58).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.02430-17.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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REFERENCES

1. McDanel J, Schweizer M, Crabb V, Nelson R, Samore M, Khader K, Blevins AE, Diekema D, Chiang HY, Nair R, Perencevich E. 2017. Incidence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* infections in the United States: a systematic literature review. Infect Control Hosp Epidemiol 38:1209–1215. https://doi.org/10.1017/ice.2017.156.
2. Lynch JP, III, Clark NM, Zhanel GG. 2013. Evolution of antimicrobial resistance among *Enterobacteriaceae* (focus on extended spectrum β-lactamases and carbapenemases). Expert Opin Pharmacother 14:199–210. https://doi.org/10.1517/14656566.2013.763030.
3. Weissman SJ, Adler A, Qin X, Zerr DM. 2013. Emergence of extended-spectrum β-lactam resistance among *Escherichia coli* at a U.S. academic children's hospital is clonal at the sequence type level for CTX-M-15, but not for CMY-2. Int J Antimicrob Agents 41:414–420. https://doi.org/10.1016/j.ijantimicag.2013.01.006.
4. Bush K. 2013. Proliferation and significance of clinically relevant β-lactamases. Ann N Y Acad Sci 1277:84–90. https://doi.org/10.1111/nyas.12023.
5. Naseer U, Sundsfjord A. 2011. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. Microb Drug Resist 17:83–97. https://doi.org/10.1089/mdr.2010.0132.
6. Gutkind GO, Di Conza J, Power P, Radice M. 2013. β-Lactamase-mediated resistance: a biochemical, epidemiological and genetic overview. Curr Pharm Des 19:164–208. https://doi.org/10.2174/138161213804070320.
7. D'Andrea MM, Arena F, Pallecchi L, Rossolini GM. 2013. CTX-M-type β-lactamases: a successful story of antibiotic resistance. Int J Med Microbiol 303:305–317. https://doi.org/10.1016/j.ijmm.2013.02.008.
8. Davis MA, Sischo WM, Jones LP, Moore DA, Ahmed S, Short DM, Besser TE. 2015. Recent emergence of *Escherichia coli* with cephalosporin resistance conferred by bla_{CTX-M} on Washington State dairy farms. Appl Environ Microbiol 81:4403–4410. https://doi.org/10.1128/AEM.00463-15.
9. Riley LW. 2014. Pandemic lineages of extraintestinal pathogenic *Escherichia coli*. Clin Microbiol Infect 20:380–390. https://doi.org/10.1111/1469-0691.12646.
10. Johnson TJ, Nolan LK. 2009. Plasmid replicon typing. Methods Mol Biol 551:27–35. https://doi.org/10.1007/978-1-60327-999-4_3.
11. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018.
12. Solà-Ginés M, Gonzalez-Lopez JJ, Cameron-Veas K, Piedra-Carrasco N, Cerda-Cuellar M, Migura-Garcia L. 2015. Houseflies (*Musca domestica*) as vectors for extended-spectrum β-lactamase-producing *Escherichia coli* on Spanish broiler farms. Appl Environ Microbiol 81:3604–3611. https://doi.org/10.1128/AEM.04252-14.
13. Medhanie GA, Pearl DL, McEwen SA, Guerin MT, Jardine CM, Schrock J, LeJeune JT. 2016. On-farm starling populations and other environmental and management factors associated with the presence of cefotaxime and ciprofloxacin resistant *E. coli* among dairy cattle in Ohio. Prev Vet Med 134:122–127. https://doi.org/10.1016/j.prevetmed.2016.10.006.
14. Feil EJ, Spratt BG. 2001. Recombination and the population structures of bacterial pathogens. Annu Rev Microbiol 55:561–590. https://doi.org/10.1146/annurev.micro.55.1.561.
15. Brabban AD, Hite E, Callaway TR. 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog Dis 2:287–303. https://doi.org/10.1089/fpd.2005.2.287.
16. Vieira AR, Collignon P, Aarestrup FM, McEwen SA, Hendriksen RS, Hald T, Wegener HC. 2011. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an ecological study. Foodborne Pathog Dis 8:1295–1301. https://doi.org/10.1089/fpd.2011.0950.
17. Kennedy D. 2013. Time to deal with antibiotics. Science 342:777. https://doi.org/10.1126/science.1248056.
18. Manges AR. 2016. *Escherichia coli* and urinary tract infections: the role of poultry-meat. Clin Microbiol Infect 22:122–129. https://doi.org/10.1016/j.cmi.2015.11.010.
19. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. 2015. Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin Infect Dis 60:439–452. https://doi.org/10.1093/cid/ciu785.
20. Abraham S, Jordan D, Wong HS, Johnson JR, Toleman MA, Wakeham DL, Gordon DM, Turnidge JD, Mollinger JL, Gibson JS, Trott DJ. 2015. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals. J Glob Antimicrob Resist 3:273–277. https://doi.org/10.1016/j.jgar.2015.08.002.
21. Belmahdi M, Bakour S, Al Bayssari C, Touati A, Rolain JM. 2016. Molecular characterisation of extended-spectrum β-lactamase- and plasmid AmpC-producing *Escherichia coli* strains isolated from broilers in Bejaia, Algeria. J Glob Antimicrob Resist 6:108–112. https://doi.org/10.1016/j.jgar.2016.04.006.
22. Eisenberger D, Carl A, Balsliemke J, Kampf P, Nickel S, Schulze G, Valenza G. 27 September 2017. Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from milk samples of dairy cows with mastitis in Bavaria, Germany. Microb Drug Resist https://doi.org/10.1089/mdr.2017.018.
23. Haenni M, Beyrouthy R, Lupo A, Chatre P, Madec JY, Bonnet R. 22 November 2017. Epidemic spread of *Escherichia coli* ST744 isolates carrying *mcr-3* and $bla_{CTX-M-55}$ in cattle in France. J Antimicrob Chemother https://doi.org/10.1093/jac/dkx418.
24. Ho PL, Cheung YY, Wang Y, Lo WU, Lai EL, Chow KH, Cheng VC. 2016. Characterization of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* from a healthcare region in Hong Kong. Eur J Clin Microbiol Infect Dis 35:379–385. https://doi.org/10.1007/s10096-015-2550-3.
25. Agabou A, Lezzar N, Ouchenane Z, Khemissi S, Satta D, Sotto A, Lavigne JP, Pantel A. 2016. Clonal relationship between human and avian ciprofloxacin-resistant *Escherichia coli* isolates in north-eastern Algeria. Eur J Clin Microbiol Infect Dis 35:227–234. https://doi.org/10.1007/s10096-015-2534-3.
26. Ben Said L, Jouini A, Alonso CA, Klibi N, Dziri R, Boudabous A, Ben Slama K, Torres C. 2016. Characteristics of extended-spectrum β-lactamase (ESBL)- and pAmpC beta-lactamase-producing *Enterobacteriaceae* of water samples in Tunisia. Sci Total Environ 550:1103–1109. https://doi.org/10.1016/j.scitotenv.2016.01.042.
27. Dissanayake DR, Octavia S, Lan R. 2014. Population structure and virulence content of avian pathogenic *Escherichia coli* isolated from outbreaks in Sri Lanka. Vet Microbiol 168:403–412. https://doi.org/10.1016/j.vetmic.2013.11.028.
28. Hasan B, Melhus A, Sandegren L, Alam M, Olsen B. 2014. The gull (*Chroicocephalus brunnicephalus*) as an environmental bioindicator and reservoir for antibiotic resistance on the coastlines of the Bay of Bengal. Microb Drug Resist 20:466–471. https://doi.org/10.1089/mdr.2013.0233.
29. Roderova M, Halova D, Papousek I, Dolejska M, Masarikova M, Hanulik V, Pudova V, Broz P, Htoutou-Sedlakova M, Sauer P, Bardon J, Cizek A, Kolar M, Literak I. 2016. Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals, and the environment in the Czech Republic. Front Microbiol 7:2147. https://doi.org/10.3389/fmicb.2016.02147.
30. Soufi L, Saenz Y, Vinue L, Abbassi MS, Hammami S, Torres C. 2013.

Characterization of Pc promoter variants of class 1 integrons in *Escherichia coli* isolates from poultry meat. Foodborne Pathog Dis 10:1075–1077. https://doi.org/10.1089/fpd.2013.1542.
31. Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A. 2006. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. Antimicrob Agents Chemother 50:3203–3206. https://doi.org/10.1128/AAC.00149-06.
32. Zurfluh K, Glier M, Hachler H, Stephan R. 2015. Replicon typing of plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ among *Enterobacteriaceae* isolated at the environment, livestock and human interface. Sci Total Environ 521–522:75–78. https://doi.org/10.1016/j.scitotenv.2015.03.079.
33. Fischer J, Rodriguez I, Baumann B, Guiral E, Beutin L, Schroeter A, Kaesbohrer A, Pfeifer Y, Helmuth R, Guerra B. 2014. $bla_{CTX\text{-}M\text{-}15}$-carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. J Antimicrob Chemother 69:2951–2958. https://doi.org/10.1093/jac/dku270.
34. Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, Nolan LK. 2007. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Appl Environ Microbiol 73:1976–1983. https://doi.org/10.1128/AEM.02171-06.
35. Johnson TJ, Siek KE, Johnson SJ, Nolan LK. 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J Bacteriol 188:745–758. https://doi.org/10.1128/JB.188.2.745-758.2006.
36. Suzuki H, Yano H, Brown CJ, Top EM. 2010. Predicting plasmid promiscuity based on genomic signature. J Bacteriol 192:6045–6055. https://doi.org/10.1128/JB.00277-10.
37. Villa L, Garcia-Fernandez A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother 65:2518–2529. https://doi.org/10.1093/jac/dkq347.
38. Day MJ, Rodriguez I, van Essen-Zandbergen A, Dierikx C, Kadlec K, Schink AK, Wu G, Chattaway MA, DoNascimento V, Wain J, Helmuth R, Guerra B, Schwarz S, Threlfall J, Woodward MJ, Coldham N, Mevius D, Woodford N. 2016. Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, the Netherlands and the UK. J Antimicrob Chemother 71:1178–1182. https://doi.org/10.1093/jac/dkv485.
39. Diab M, Hamze M, Madec JY, Haenni M. 2017. High prevalence of non-ST131 CTX-M-15-producing *Escherichia coli* in healthy cattle in Lebanon. Microb Drug Resist 23:261–266. https://doi.org/10.1089/mdr.2016.0019.
40. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18:646–655. https://doi.org/10.1111/j.1469-0691.2012.03850.x.
41. Liebana E, Batchelor M, Hopkins KL, Clifton-Hadley FA, Teale CJ, Foster A, Barker L, Threlfall EJ, Davies RH. 2006. Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. J Clin Microbiol 44:1630–1634. https://doi.org/10.1128/JCM.44.5.1630-1634.2006.
42. Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Wearing H, Duncan D, Rabie A, McLaren I, Watson E, La Ragione RM, Coldham NG. 2011. Fecal carriage and shedding density of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* in cattle, chickens, and pigs: implications for environmental contamination and food production. Appl Environ Microbiol 77:3715–3719. https://doi.org/10.1128/AEM.02831-10.
43. Tenaillon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol 8:207–217. https://doi.org/10.1038/nrmicro2298.
44. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Herman L, Haesebrouck F, Butaye P. 2010. Broad-spectrum β-lactamases among *Enterobacteriaceae* of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiol Rev 34:295–316. https://doi.org/10.1111/j.1574-6976.2009.00198.x.
45. Bevan ER, Jones AM, Hawkey PM. 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145–2155. https://doi.org/10.1093/jac/dkx146.
46. Wittum TE, Mollenkopf DF, Daniels JB, Parkinson AE, Mathews JL, Fry PR, Abley MJ, Gebreyes WA. 2010. CTX-M-type extended-spectrum β-lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. Foodborne Pathog Dis 7:1575–1579. https://doi.org/10.1089/fpd.2010.0615.
47. Bej AK, DiCesare JL, Haff L, Atlas RM. 1991. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for *uid*. Appl Environ Microbiol 57:1013–1017.
48. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. 2003. Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob Agents Chemother 47:3724–3732. https://doi.org/10.1128/AAC.47.12.3724-3732.2003.
49. Ahmed S, Besser TE, Call DR, Weissman SJ, Jones LP, Davis MA. 2016. Evaluation of two multi-locus sequence typing schemes for commensal *Escherichia coli* from dairy cattle in Washington State. J Microbiol Methods 124:57–61. https://doi.org/10.1016/j.mimet.2016.03.008.
50. Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standards—4th ed. VET01-A4. Clinical and Laboratory Standards Institute, Wayne, PA.
51. Lewis JS, II, Herrera M, Wickes B, Patterson JE, Jorgensen JH. 2007. First report of the emergence of CTX-M-type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother 51:4015–4021. https://doi.org/10.1128/AAC.00576-07.
52. Ruppé E, Hem S, Lath S, Gautier V, Ariey F, Sarthou JL, Monchy D, Arlet G. 2009. CTX-M beta-lactamases in *Escherichia coli* from community-acquired urinary tract infections, Cambodia. Emerg Infect Dis 15:741–748. https://doi.org/10.3201/eid1505.071299.
53. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
54. Kado CI, Liu ST. 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145:1365–1373.
55. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
56. Abramson JH. 2011. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov 8:1. https://doi.org/10.1186/1742-5573-8-1.
57. Hunter PR, Gaston MA. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26:2465–2466.
58. Grundmann H, Hori S, Tanner G. 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J Clin Microbiol 39:4190–4192. https://doi.org/10.1128/JCM.39.11.4190-4192.2001.
59. Pitout JD, Hossain A, Hanson ND. 2004. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp. J Clin Microbiol 42:5715–5721. https://doi.org/10.1128/JCM.42.12.5715-5721.2004.
60. Eckert C, Gautier V, Arlet G. 2006. DNA sequence analysis of the genetic environment of various $bla_{CTX\text{-}M}$ genes. J Antimicrob Chemother 57:14–23. https://doi.org/10.1093/jac/dki398.