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Transmission mechanisms of Extended Spectrum Beta-Lactamase (ESBL) genes among human and domestic animals in Ecuador

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“He who learns without thinking will be bewildered;

he who thinks without learning will be in danger.”

Confucius

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RESUMEN

La resistencia antimicrobiana (RAM) constituye una amenaza a la salud pública mundial pero especialmente en países de ingresos bajos y medianos debido al uso excesivo de antimicrobianos, falta de aplicación de regulaciones de venta de antimicrobianos, condiciones de saneamiento precarias y hacinamiento. El uso de antimicrobianos en producción animal para consumo intensifica la propagación de RAM, particularmente en sistemas de pequeña escala, donde existe un contacto estrecho entre humanos y animales, así como con sus desechos. La rápida diseminación de *Escherichia coli* productora de β-lactamasa de espectro extendido portadora del gen $bla_{\text{CTX-M}}$ es de particular preocupación, ya que esta resistencia se está detectando progresivamente en entornos clínicos como comunitarios a nivel mundial. En este contexto, el rol de los animales en la transmisión de RAM no es claro, debido a estudios contradictorios sobre la transmisión de *E. coli* resistente a antimicrobianos de animales a humanos. Estas discrepancias probablemente se deben a esquemas de muestreo inadecuados, los que subestima la diversidad y altas tasas de renovación de cepas *E. coli* en una comunidad, así como a la ubiquidad de genes de RAM antiguos que se han diseminado globalmente durante décadas en las poblaciones bacterianas. El objetivo de esta investigación doctoral fue determinar los mecanismos de transmisión de *E. coli* resistente a cefalosporinas de tercera generación a partir de muestras fecales de niños y animales domésticos recolectadas en una comunidad ecuatoriana durante el mismo periodo. Se investigó la relación genotípica de estas cepas mediante secuenciación de genoma completo y se caracterizó los plásmidos portadores del gen $bla_{\text{CTX-M}}$, para evaluar la contribución de la diseminación de cepas resistentes y la transferencia horizontal de genes en la transmisión de RAM.

Palabras clave: *Escherichia coli*, resistencia antimicrobiana, comunidad, relación clonal, transferencia horizontal de genes, $bla_{\text{CTX-M}}$

ABSTRACT

Antimicrobial resistance (AMR) is an urgent global public health threat that affects predominantly to low and middle-income countries (LMICs) due to antimicrobial overuse, poor sanitation, lack of enforcement of laws restricting the free purchase of antimicrobials, and overcrowding. The indiscriminate use of antimicrobials in food-animal production intensifies AMR spread, especially within small-scale systems where humans have close contact with domestic animals and their waste. The rapid dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* carrying the $bla_{CTX\text{-}M}$ gene is of particular concern as it is being detected increasingly in clinical and community settings. In this context, the role of animals in AMR dissemination remains unclear, with conflicting studies on the transmission of antimicrobial-resistant *E. coli* from domestic animals to humans. This discrepancy is probably due to inadequate sampling schemes that underestimate the diversity and the high turnover rates of *E. coli* strains in a community and the detection of old AMR genes that have been disseminated globally for decades. This doctoral research aimed to assess the transmission mechanisms of third-generation cephalosporin-resistant *E. coli* (3GCR-EC) from temporally and spatially matched fecal samples collected from children and domestic animal feces present in the household environment in Ecuadorian semirural communities. We investigate the genotypic relationship of 3GCR-EC strains using whole genome sequencing (WGS) and we characterize plasmids carrying $bla_{CTX\text{-}M}$ gene, harbored by these 3GCR-EC strains to assess the contribution of clonal transmission and horizontal gene transfer (HGT) in the spread of AMR.

Key words: *Escherichia coli*, antimicrobial resistance, community, clonal relationship, horizontal gene transfer, $bla_{CTX\text{-}M}$.

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CHAPTER 1

Introduction

Bacterial antimicrobial resistance (AMR) has emerged as one of the leading public health threats affecting human, animal and environmental health, and represents a significant cost for the global economy (Lim et al. 2016; Redfield 2019; World Health Organization 2023). AMR caused around 1.27 million deaths globally in 2019 (Murray et al. 2022) and it is estimated that AMR could cause 10 million deaths per year by 2050 (O`Neill 2016), affecting low and middle-income countries (LMICs) more severely (Alvarez-Uria et al. 2016; Ashley et al. 2018; O`Neill 2016; Pearson and Chandler 2019). In LMICs, antimicrobial overuse and consequent AMR have higher development rates than in high-income countries due to several reasons, including lack of regulation on antimicrobial sales and use, self-medication, (Alvarez-Uria et al. 2016; Ashley et al. 2018; Lim et al. 2016; Pearson and Chandler 2019), poor access to sanitation and hygiene infrastructure (Alvarez-Uria et al. 2016; Ashley et al. 2018; O`Neill 2016; Pearson and Chandler 2019), inadequate cleanliness and overcrowding (Sharma et al. 2022).

Additionally, the growing spread of AMR have been associated with the indiscriminate use of antimicrobials in veterinary medicine (Argudín et al. 2017; Hao et al. 2016), and food animal production, mostly as growth promoters or prophylactics (Barton et al. 2003; Bush et al. 2011; Marshall and Levy 2011; Subbiah et al. 2020; Van Boeckel et al. 2019). In LMICs, small-scale systems where typically raise animals at lower density within the household environment and to roam freely are common (Penakalapati et al. 2017). These settings with lack management practices, that include direct contact with animals and their wastes are considered important reservoir for the transmission of antimicrobial-resistant bacteria and AMR genes among animals and humans, especially among children (Graham et al. 2017).

Antimicrobial resistance profiles in *Escherichia coli* (probably the most used AMR indicator) isolated from humans and animals have revealed significant changes during the years 1950 to 2002 (Tadesse et al. 2012). The prevalence of multidrug-resistant *E. coli* isolates, resistant to at least one agent in three or more antimicrobial classes (Magiorakos et al. 2012), increased from 7.2% in the 1950s to 63.6% in the 2000s (Tadesse et al. 2012). Selective pressure exerted by antimicrobial use has been the major driving force behind the emergence and spread of AMR (Aarestrup et al. 2008). In fact, resistance has developed after the emergence of every major class of antimicrobial, varying from one to more than ten years (Levy and Bonnie 2004). For instance, resistance to sulfonamide has been one of the most common resistances identified in human and animal *E. coli* isolates since 1950 and 1964, respectively, after its use began in the 1930s (Tadesse et al. 2012).

Overall, the most common AMR phenotypes in human and animal *E. coli* isolates from 1950 to 2002 were to older antimicrobials such as tetracycline (introduced in 1948), sulfonamide (introduced in 1936), streptomycin (introduced in 1943), and ampicillin (introduced in 1961) (Tadesse et al. 2012). Whereas a scarce number of isolates were resistant to antimicrobials introduced for clinical use since 1980, such as amoxicillin/clavulanic acid (introduced in 1984), ceftriaxone (introduced in 1984) and ciprofloxacin (introduced in 1987). Interestingly, persistence of resistance to older antimicrobials has been reported despite a major reduction in the rate of the antimicrobials use (Bean et al. 2005; Langlois et al. 1983; Tadesse et al. 2012) and these resistance traits are presently extensively spread within *E. coli* populations (Ingle et al. 2018; Johnson et al. 2012; Salinas et al. 2019).

In this context, the dramatic dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales has been of major concern, especially of *E. coli* carrying CTX-M

enzyme, the most common specie associated with global ESBLs (Livermore et al. 2007; Peirano and Pitout 2019). The prevalence of CTX-M enzymes has increased rapidly since the mid-late 2000s (Peirano and Pitout 2019) and, unlike many acquired ESBL which origin remains unknown, it has been attributed that *bla*$_{\text{CTX-M}}$ genes have their origin in chromosomes of several species of *Kluyvera* genus (Cantón et al. 2008; D'Andrea et al. 2013; Woerther et al. 2013).

In recent years, ESBL-producing *E. coli,* resistant to third- or later-generation cephalosporins (Zamudio et al. 2022), has been found in large number of clinical settings (Branger et al. 2018; Cadena et al. 2020; Doi et al. 2017; Zhao et al. 2013) probably due to antimicrobials pressure. However, ESBL-producing *E. coli* has also been identified in domestic animals and humans at community level around the world (Benavides et al. 2021; Doi et al. 2017; Wei et al. 2022).

Despite, the role of animals in the dissemination and spread of AMR is unclear (Graham et al. 2017). The role of transmission of antimicrobial-resistant *E. coli* from domestic animals to humans in the current crisis of AMR has been controversial; some argue for high importance (Berg et al. 2017; Borges et al. 2019; Dorado-García et al. 2018; Johnson and Clabots 2006; Marshall and Levy 2011; Pietsch et al. 2018), others challenged this notion (Day et al. 2019; de Been et al. 2014; Ludden et al. 2019). In LMICs the data on AMR and antimicrobials use in humans and animals is limited or lacking (Tiseo et al. 2020), making it difficult to establish their true impact in human health (Ikhimiukor and Okeke 2023).

Taken together, we hypothesize that contradictory results on transmission of antimicrobial-resistant bacteria and AMR genes among domestic animals and humans are caused by sampling schemes that underestimate the diversity and the high turnover rates of

E. coli strains in a community and the detection of old AMR genes that are disseminated globally, unlike AMR genes as those encoding resistance to third-generation cephalosporin that are preferentially present in *E. coli* population that have been under antimicrobial pressure. We hypothesized that the household environment, where the feces of domestic animals are deposited, serves as a reservoir of ESBL-producing *E. coli* and that children are subsequently exposed to those same isolates.

Therefore, the objective of the present doctoral research was asses the transmission mechanisms of third-generation cephalosporin-resistant *E. coli* (3GCR-EC) from temporally and spatially matched fecal samples collected from children and domestic animal feces present in the household environment in semirural communities of Ecuador. We investigate the genotypic relationship of 3GCR-EC strains using whole genome sequencing (WGS) and we characterize mobile genetic elements (MGEs) carrying $bla_{\text{CTX-M}}$ gene, harbored by these 3GCR-EC strains to assess the contribution of clonal transmission and HGT in the spread of AMR.

The results of this research are presented in two chapters:

- **Chapter 2:** Environmental Spread of Extended Spectrum Beta-lactamase (ESBL) Producing *Escherichia coli* and ESBL Genes Among Children and Domestic Animals in Ecuador. *Published: Environmental Health Perspectives, 2021. Volume 129, Issue 2. CID: 027007. https://doi.org/10.1289/EHP77.*
- **Chapter 3**: IS26 drives the dissemination of $bla_{\text{CTX-M}}$ genes in an Ecuadorian community. *Publication: Microbiology Spectrum, 2023. Month January/February 2024, Volume 12. Issue 1. https://doi.org/10.1128/spectrum.02504-23.*

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CHAPTER 2

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Environmental Spread of Extended Spectrum Beta-lactamase (ESBL) Producing *Escherichia coli* and ESBL Genes Among Children and Domestic Animals in Ecuador

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BACKGROUND: There is a significant gap in our understanding of the sources of multidrug-resistant bacteria and resistance genes in community settings where human–animal interfaces exist.
OBJECTIVES: This study characterized the relationship of third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolated from animal feces in the environment and child feces based on phenotypic antimicrobial resistance (AMR) and whole genome sequencing (WGS).
METHODS: We examined 3GCR-EC isolated from environmental fecal samples of domestic animals and child fecal samples in Ecuador. We analyzed phenotypic and genotypic AMR, as well as clonal relationships (CRs) based on pairwise single-nucleotide polymorphisms (SNPs) analysis of 3GCREC core genomes. CRs were defined as isolates with fewer than 100 different SNPs.
RESULTS: A total of 264 3GCR-EC isolates from children (n= 21), dogs (n= 20), and chickens (n= 18) living in the same region of Quito, Ecuador, were identified. We detected 16 CRs total, which were found between 7 children and 5 domestic animals (5 CRs) and between 19 domestic animals (11 CRs). We observed that several clonally related 3GCR-EC isolates had acquired different plasmids and AMR genes. Most CRs were observed in different homes (n= 14) at relatively large distances. Isolates from children and domestic animals shared the same $bla_{\text{CTX-M}}$ allelic variants, and the most prevalent were $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$, which were found in isolates from children, dogs, and chickens.
DISCUSSION: This study provides evidence of highly dynamic horizontal transfer of AMR genes and mobile genetic elements (MGEs) in the *E. coli* community and shows that some 3GCR-EC and (extended-spectrum b-lactamase) ESBL genes may have moved relatively large distances among domestic animals and children in semirural communities near Quito, Ecuador. Child animal contact and the presence of domestic animal feces in the environment potentially serve as important sources of drug-resistant bacteria and ESBL genes.

Introduction

Antimicrobial resistance (AMR) constitutes one of the biggest public health threats affecting not only human and animal health, but also the global economy (Lim et al. 2016; Redfield 2019; WHO 2018). More than 2.8 million infections caused by drug-resistant bacteria have resulted in more than 35,000 annual deaths in the United States (CDC 2019), and 33,000

annual deaths were estimated for the European Union (Plachouras et al. 2018). Low- and middle-income countries (LMICs) face the greatest burden of AMR (Alvarez-Uria et al. 2016; Ashley et al. 2018; Pearson and Chandler 2019) because of poor sanitation and hygiene infrastructure and lack of regulation on antimicrobial sales and use (Alvarez-Uria et al. 2016; Ashley et al. 2018; Lim et al. 2016; Pearson and Chandler 2019; Robinson et al. 2016).

The rapid emergence and spread of AMR have been associated with the heavy use of antimicrobials in human medicine (IACG 2019), veterinary medicine (Argudín et al. 2017; Hao et al. 2016), and food animal production (Marshall and Levy 2011; Van Boeckel et al. 2015). Currently, 73% of all antimicrobials sold in the world are estimated to be used in food animals (Van Boeckel et al. 2019), mostly as growth promoters or prophylactics (Barton et al. 2003; Bush et al. 2011; Subbiah et al. 2020; Van Boeckel et al. 2015, 2019). In LMICs, a large number of small-scale animal operations lack appropriate animal-waste management (Lowenstein et al. 2016; Penakalapati et al. 2017), and domestic animals (carrying antimicrobial-resistant bacteria) are allowed to roam freely, contaminating households, soil and irrigation channels (Penakalapati et al. 2017). This environment can then act as a reservoir of drug-resistant bacteria, AMR genes, antibiotics and other agents (Ashbolt et al. 2018) that can spread among humans and domestic animals (Ashbolt et al. 2018; Borges et al. 2019; Penakalapati et al. 2017). Despite this, the role of animals and animal waste in the global AMR crisis is poorly understood and controversial (Graham et al. 2017).

Evidence from observational studies shows that AMR in bacteria from domestic animals is transmitted to intestinal bacteria in humans (Berg et al. 2017; Borges et al. 2019; Dorado-García et al. 2018; Johnson and Clabots 2006; Marshall and Levy 2011; Pietsch et al. 2018). However, recent observational studies using whole genome sequencing (WGS) and

focusing on extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli,* have challenged this notion (Day et al. 2019; de Been et al. 2014; Ludden et al. 2019). We hypothesize that contradictory results are caused by sampling schemes that underestimate the diversity and the high turnover rates of *E. coli* strains in a community. In this study, we investigated the genotypic relationship of third-generation cephalosporin-resistant *E. coli* (3GCR-EC) using WGS. In contrast to previous studies, we isolated *E. coli* from temporally and spatially matched fecal samples collected from young children and domestic animal feces present in the household environment in semirural communities in Ecuador. We hypothesized that the household environment where the feces of domestic animals are deposited serves as a reservoir of 3GCR-EC and that children are subsequently exposed to those same isolates.

Materials and Methods

Study location

This study was part of a larger research project (374 households) that was conducted in semirural communities of six parishes located to the northeast of Quito, Ecuador, to assess the role of social and environmental factors, and knowledge, attitude and practices (KAP) of use of antibiotics in the transmission of 3GCR-EC and ESBL genes among domestic animals and humans. In these communities, small-scale domestic animal production is common. We stratified the study area into geographic quandrants using satellite imagery and each quandrant was assigned a random number (using a random numbers table). Households were enrolled in each selected quandrant if they met the following inclusion criteria: *a*) there was a primary child care provider present who was over 18 years of age; *b*) there was a child between the ages of 6 months and 4 y; and *c*) an informed consent was provided by a primary

child care provider to participate in the study. Among the households studied, we conducted an additional stratification step to select 10 households without domestic animals where a child was positive for presumptive 3GCR-EC and 19 households with domestic animals where a child was positive for presumptive 3GCR-EC to include for the phenotypic and genotypic analysis. Children and domestic animal stool samples were collected at the same time. This stratification resulted in 66% of households (19 out of 29) with dogs and chickens and 34% (10 out of 29) with no domestic animals, a distribution of households that reflected the overall makeup of the studied communities, in which approximately two-thirds had domestic animals (Marusinec et al. 2021). The geographical coordinates for each household were obtained. Fecal samples from 29 young children (between the ages of 6 months and 4 y) were collected, as well as 39 fecal samples from domestic animals (20 dogs and 19 chickens) that were present in the household environment.

Ethical considerations

The study was approved by Committee for Protection of Human Subjects (CPHS) and the Office for Protection of Human Subjects (OPHS) at the University of California-Berkeley (Federalwide Assurance #6252) and by the Bioethics Committee at the Universidad San Francisco de Quito (2017-178IN).

Household survey

Primary child care providers were interviewed outside of their home applying a household survey that covered questions about demographics; domestic animal and child antimicrobial

use; water, sanitation, and hygiene (WaSH) conditions; and animal ownership (Table 1 and Table 2).

Table 1. Characteristics of children, household members, and water, sanitation, and hygiene (WaSH) conditions in study households

Household and child characteristics	n = 29 (100%)
Parish	
1	12 (41.4)
2	6 (20.7)
3	6 (20.7)
4	1 (3.4)
5	2 (6.9)
6	2 (6.9)
Child sex[a]	
Female	16 (55.2)
Male	13 (44.8)
Child age[a]	
< 1 year old	6 (20.7)
1 year old	8 (27.6)
2 years old	6 (20.7)
3 years old	7 (24.1)
4 years old	2 (6.9)
Primary caregiver education level	
Elementary	9 (31.0)
High school	15 (51.2)
College	5 (17.2)
No. people living in household	
1-2	0 (0)
3-4	16 (55.2)
5-6	12 (41.4)
7-8	1 (3.4)
Household sanitation facility	
Toilet that flushes into sewer	26 (89.7)
Toilet with septic system	3 (10.3)
Household main source of drinking water	
Tap water inside the house	21 (72.4)
Tap water outside the house	4 (13.8)
Public tap	1 (3.4)
Bottled wáter	1 (3.4)
Don't know	2 (6.9)
Household water treatment method	
No treatment	15 (51.7)
Boil	11 (37.9)
Other	3 (10.3)
Household handwashing facility	
Soap & water present	26 (89.7)
Water only	1 (3.4)
Neither	2 (6.9)
Child feces disposal	
Placed in toilet	13 (44.8)
Placed in waste bin	16 (55.2)
Child administered antibiotics in last 3 months	
No	23 (79.3)
Yes	6 (20.7)

[a]Refers to the child enrolled in the study.

Table 2. Characteristics of domestic animal ownership in study households

Household animal characteristics	n = 29 (100%)
No. household animals owned	
0	10 (34.5)
1-10	8 (27.6)
11-20	3 (10.3)
20-40	5 (17.2)
40-60	1 (3.4)
60-100	0 (0)
101-125	2 (6.9)
No. dogs owned	
0	10 (34.5)
1-2	14 (48.3)
3-5	3 (10.3)
6-10	1 (3.4)
11-12	1 (3.4)
No. chickens owned	
0	10 (34.5)
1-5	9 (31.0)
6-10	4 (13.8)
11-25	5 (17.2)
26-50	0 (0)
51-100	1 (3.4)
Other animals owned	
Pigs	3 (10.3)
Cows	3 (10.3)
Guinea pigs	8 (27.6)
Ducks	4 (13.8)
Goats or sheep	2 (6.9)
Cats	6 (20.7)
Domestic animal feces disposal	
Left in yard to decompose	8 (27.6)
Used in crops as fertilizer	8 (27.6)
Placed in waste bin	2 (6.9)
Don't know	1 (3.4)
Doesn't apply (no animals)	10 (34.5)
Distance to nearest commercial food-animal production facility	
< 0.5 km	3 (10.3)
0.5-1 km	6 (20.7)
1-1.5 km	7 (24.1)
1.5-2 km	6 (20.7)
2+ km	7 (24.1)
No. commercial food-animal production facilities within 5 km	
0	2 (6.9)
1-5	8 (27.6)
6-10	7 (24.1)
11-20	7 (24.1)
> 20	5 (17.2)
Household animals administered antibiotics in last 6 months	
No	25 (86.2)
Yes	4 (13.8)

The household survey included the child's interactions with domestic animals, exposures to food-animal production and domestic animal handling characteristics (Table 3). Interviews took approximately 25 min to complete at enrollment and were conducted by trained staff. Descriptive statistics were performed using R (version 4.0.2; R Development Core Team) and the package tableone (Yoshida et al. 2020).

Sample collection

In each household, a single stool sample was collected from a child and from chickens and dogs living in the children's households from August to November 2018. If more than one child (ages of 6 months and 4 y) resided in the same household, field staff selected the younger child to participate in the study. Stool samples from children were collected by their primary caretaker using a fecal collection kit provided by the study team. Caregivers were instructed about how to collect child stool samples avoiding contact with diaper or toilet bowl, as described previously (Salinas et al. 2019). Participants were instructed to double-bag the sample container and keep it in the refrigerator until field staff could pick up the sample the same morning. Simultaneously, fresh dog and chicken fecal samples (i.e., visual evidence of high moisture content) were collected from the household outdoor environment where the animals commonly defecated. Field staff used a single-use glove to collect the sample and attempted to avoid any additional contamination (i.e., soil). If more than one dog or chicken were living in a household, field staff collected fecal matter from a single deposit representing the feces of one animal. The samples were placed in sterile containers and transported on ice packs at approximately 4°C to the laboratory and were processed within 5 h of collection.

Table 3. Domestic animal handling practices, child contact with animals, and exposures to food-animal production

	Overall	Household Animal Ownership	
	n = 29 (100%)	No animals n = 10 (34.5%)	Animals n = 19 (65.5%)
Animals allowed inside home			
No	20 (69.0)	10 (50.0)	10 (50.0)
Yes	9 (31.0)	0 (0)	9 (100)
Frequency of child contact with poultry in last 3 months			
Never	15 (51.2)	9 (60.0)	6 (40.0)
< 1 time per week	0 (0)	0 (0)	0 (0)
1-2 times per week	3 (10.3)	1 (33.3)	2 (66.7)
3 times or more per week	11 (37.9)	0 (0)	11 (100)
Frequency of child contact with pets in last 3 months			
Never	11 (37.9)	8 (72.7)	3 (27.3)
< 1 time per week	2 (6.9)	0 (0)	2 (100)
1-2 times per week	4 (13.8)	1 (25.0)	3 (75.0)
3 times or more per week	12 (41.4)	1 (8.3)	11 (91.7)
Animals entered area where child spends time in last 3 weeks			
No	19 (65.5)	10 (52.6)	9 (47.4)
Yes	10 (34.5)	0 (0)	10 (100)
Child played in area where animals defecate in last 3 weeks			
No	18 (62.1)	10 (55.6)	8 (44.4)
Yes	11 (37.9)	0 (0)	11 (100)
Frequency of child contact with pets or poultry in last 3 weeks			
Never	12 (41.4)	10 (83.3)	2 (16.7)
< 1 time per week	1 (3.4)	0 (0)	1 (100)
1-2 times per week	4 (13.8)	0 (0)	4 (100)
3 times or more per week	12 (41.4)	0 (0)	12 (100)
Child washes hands after contact with animals			
Never	1 (4.0)	0 (0)	1 (100)
Rarely	1 (3.4)	4 (22.2)	14 (77.8)
Sometimes	5 (20.0)	2 (40.0)	3 (60.0)
Always	18 (62.1)	0 (0)	1 (100)
Refused to answer	4 (13.8)	4 (100)	0 (0)
Household member worked with animals outside the home in last 6 months			
No	28 (96.6)	10 (35.7)	18 (64.3)
Yes	1 (3.4)	0 (0)	1 (100)
Household member worked in processing of food-animal products in last 6 months			
No	17 (58.6)	9 (52.9)	8 (47.1)
Yes	12 (41.4)	1 (8.3)	11 (91.7)
Household member handled human or animal feces outside the home in last 6 months			
No	27 (93.1)	10 (37.0)	17 (63.0)
Yes	2 (6.9)	0 (0)	2 (100)

Note: All households that reported owning animals reported owning both chickens and dogs.

Isolation of 3GCR-EC

Fecal samples were plated onto MacConkey agar (Difco) supplemented with ceftriaxone (2 mg/L), a third-generation cephalosporin (3GC) (Botelho et al. 2015) and incubated overnight at 37°C, after which five lactose-positive colonies were selected (Lautenbach et al. 2008). *E. coli* ATCC 25922 (American Type Culture Collection) was used as negative control for presumptive 3GC-resistant isolates. The identity of presumptive *E. coli* colonies was confirmed by culture on Chromocult coliform agar (Merck KGaA), at 37°C for 24 h, through its b-D-glucuronidase activity (Lange et al. 2013), followed by the multisubstrate API RapiD-20E identification system (bioMérieux) using a cut off of 95%. All confirmed 3GCR-EC isolates from each sample were kept frozen at -80°C in Tryptic Soy Broth medium (Difco) with 15% glycerol.

Antimicrobial susceptibility testing

Each 3GCR-EC isolate was reactivated on MacConkey agar supplemented with ceftriaxone (2 mg/L), at 37°C for 18 h. Antimicrobial susceptibility testing for all isolates was performed by the disk diffusion method using Mueller-Hinton agar (Difco). Antibiogram plates were incubated at 37°C for 18 h according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2018). *E. coli* ATCC 25922 was used as a reference strain. Antimicrobials (BD BBL Sensi-Disc) used included the following: amoxicillin-clavulanate (AMC; 20 per 10 micrograms), ampicillin (AM; 10 µg), cefazolin (CZ; 30 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), cefepime (FEP; 30 µg), chloramphenicol (C; 30 µg), ciprofloxacin (CIP; 5 µg), gentamicin (GM; 10 µg), imipenem (IPM; 10 µg), tetracycline (TE; 30 µg), and trimethoprim-sulfamethoxazole (SXT; 1.25 per 23.75 micrograms) (CLSI 2018).

DNA sequencing and analysis

Genomic DNA was extracted from the isolates using the Wizard® Genomic DNA Purification (Promega) according to the manufacturer's instructions. The whole genome of isolates was sequenced using Illumina MiSeq. Sequencing was carried out at the University of Minnesota Mid-Central Research and Outreach Center (Willmar, Minnesota) using a single 2X250-bp dual-index run on an Illumina MiSeq with Nextera XT libraries to generate approximately 30- to 50-fold coverage per genome. Illumina raw reads were quality-trimmed and adapter-trimmed using trimmomatic (Bolger et al. 2014). Genome assembly of Miseq reads for each sample was performed using SPAdes assembler with the careful assembly option and automated k-mer detection (Bankevich et al. 2012). Acquired AMR genes, plasmid types and serotypes were identified using ABRicate tool (version 0.8.13), comparing the whole genomes against in-house curated versions of the Resfinder database for resistance gene identification (Zankari et al. 2012), with 90% minimum match and 60% minimum length; PlasmidFinder database for plasmid replicon identification (Carattoli et al. 2014), with 95% minimum match and 60% minimum length; and EcOH database for O serogroup and H flagellar antigen detection (Ingle et al. 2016), with 85% minimum match and 60% minimum length. Differences among ESBL-encoding $bla_{\text{CTX-M}}$ gene variants of isolates from children, dogs and chickens were tested with a chi-square test ($p < 0.05$) using chisq.test function in R (version 3.6.2; R Development Core Team).

Phylogenetic analysis

Assembled genome contigs were mapped to the *E. coli* O157:H7 reference genome (GenBank accession no. NC_002695) using Mauve (Darling et al. 2011). Pan-genome analysis was carried

out using Roary (Page et al. 2015); core genes were defined as genes being in at least 99% of isolates analyzed. A maximum-likelihood phylogenetic tree with 1,000 bootstrap replicates based on core genomes of isolates was created using RaxML-NG (Kozlov et al. 2019). For phylogenetic tree construction, isolates with more than 100 differences in pairwise single nucleotide polymorphisms (SNPs) analysis in the core genome were selected from each individual; if two or more isolates had fewer than 100 SNPs, one was selected randomly. The phylogenetic tree was visualized using iTOL (Letunic and Bork 2019). Clonal relationships (CRs) were arbitrarily defined as two or more *E. coli* isolates having fewer than 100 SNPs in the core genome using Snippy software (version 4.3.9). Clonal relationships were defined based on core genomes obtained from WGS, which provides ample discriminatory power to provide evidence of transmission or close relatedness among isolates. We used WGS because it is not subject to artifacts such as homoplasy where sequence types (STs) may share similarities but do not arise by recent common ancestry (Pietsch et al. 2018) or isolates belonging to same ST but having several SNP differences in their core genomes (Salinas et al. 2019) and therefore no evidence of recent ancestry. Euclidean distance between households of hosts involved in each CR was calculated using R packages ggmap (Kahle and Wickham 2013) and kableExtra (version 1.1.0). Additionally, an *in silico* multilocus sequence typing (MLST), based on seven housekeeping genes (*adk, fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*), additional eight housekeeping genes (*dinB*, *icdA*, *pabB*, *polB*, *putP*, *trpA*, *trpB*, and *uidA*), and core genome (cgMLST) was performed using MLST 2.0 (Larsen et al. 2012), and cgMLSTFinder 1.1 (Alikhan et al. 2018), tools available through the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/). Phylogenetic groups were assigned using *in silico* ClermonTyping 1.4.1 (Beghain et al. 2018).

Accession number(s)

Assembled genome contigs have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB37285 (https://www.ebi.ac.uk/ena/data/view/PRJEB37285).

Results

Two hundred ninety-four 3GC-resistant isolates were obtained from 68 fecal samples (children = 29, dogs = 20, chickens = 19) collected in 29 households, of which 19 had dogs and chickens, and 10 had no domestic animals. All households that reported owning any animals reported owning both dogs and chickens (Table S1). Characteristics of household members, domestic animal ownership, and WaSH conditions in study households are shown in Table 1 and Table 2, whereas domestic animal handling practices, child contact with animals, and exposures to food-animal production are shown in Table 3. Of the 294 isolates, 264 were 3GCR-EC isolates from 21 children (n = 93 isolates), 20 dogs (n = 92 isolates) and 18 chickens (n = 79 isolates).

Clonal Relationships Among 3GCR-EC Isolates

Core genomes of the isolates showed that some *E. coli* clonal relationships were shared by different animal species: 1 CR was shared by a child and a dog, 3 CRs were shared by 3 pairs of child-chicken (one of them formed by a child and a chicken from the same household), 1 CR among 3 children and a dog (1 child and a dog from the same household), 3 CRs shared between 3 pairs of dog-chicken (one of them formed by a dog and a chicken from the same household). Some CRs were shared by the same animal species: 2 CRs between 2 pairs of dogs,

4 CRs between 4 pairs of chickens, 1 CR among 3 dogs, and 1 CR among 3 chickens (Figure 1 and Figure 2). The number of SNPs for each pairwise analysis is shown in Tables S2-S17. A total of 28 individuals across all three species: dogs (*n* = 11), chickens (*n* = 10), and children (*n* = 7) from 58.6% (*n* = 17) of study households were involved in the 16 CRs identified. Two children from households with no domestic animals had 2 CRs that were linked to children and domestic animals from different households (Table S3 and Table S9). It is interesting, to note that, for a child involved in CR B (3 children and a dog), the caregiver reported that the child had contact with pets at a frequency of 3 or more times per week in the last 6 months previous to enrollment in the study, whereas for the child involved in CR H (1 child and a dog) the caregiver reported that the child had no contact with pets or poultry in the same period of time (Excel Table S1). The surveys of the 17 households involved in CRs showed that most households had access to sanitation and water: *a*) had a toilet facility connected to sewer lines (*n* = 15, 88.2%); *b*) child feces were placed in the toilet (*n* = 13, 76.5%); *c*) main source of drinking water was tap water inside the house (*n* = 11, 61.7%). Similarly, most households had good hygiene practices: *a*) child was reported to wash hands after contact with animals (*n* = 14, 82.4%); *b*) the handwashing facility had soap and water available (*n* = 16, 94.1%); *c*) animals were not allowed inside the home (*n* = 10, 58.8%); *d*) animals did not enter area where child spends time (*n* = 9, 52.9%). In contrast, in most households the management of domestic animal fecal waste and handling practices were problematic: *a*) domestic animals feces were left in the yard to decompose or used on crops as fertilizer (*n* = 14, 82.4%); *b*) child played in area where animals defecated (*n* = 10, 58.8%); and *c*) the child had contact with animals (*n* = 15, 88.2%). Additionally, occupational risks in most households were low: *a*) many household members did not work in processing of food-animal products (*n* = 10, 58.8%); *b*) most

household members did not work with animals outside the home (*n* = 16, 94.1%); and *c*) most household members did not handle human or animal feces outside the home (*n* = 15, 88.2%) (Excel Table S1).

Clonal relationships of 3GCR-EC were identified among samples collected throughout the study area (Figure 2). We found three households where the same CRs were identified at the same household (Euclidean distance = 0 km; Figure 1). However, the distance between individuals in CRs ranged from 0 to nearly 9 km (median = 2.7), and 25% of pairs were at least 4.7 km apart. Individuals in CR B, for example, included a dog and child from the same household, as well as two other children from different households up to 5.6 km away. Additionally, CR J included 3 chickens up to 2.7 km apart, and CR P included 3 dogs up to almost 9 km apart (Figure 1).

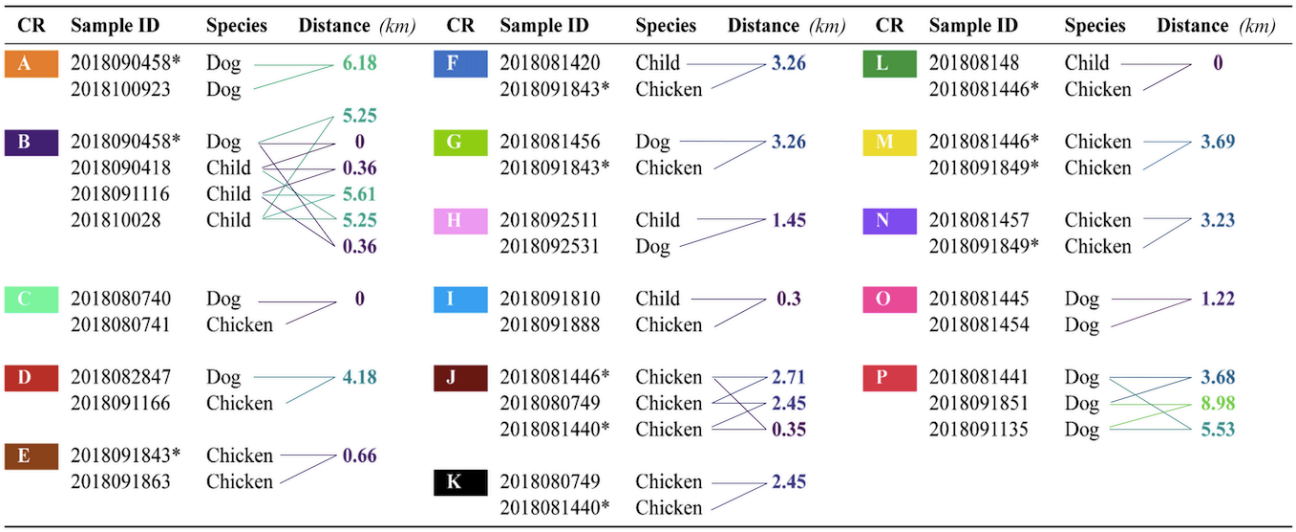

Figure 1. Euclidean distance (in kilometers) between host samples with clonal relationships (CRs) of third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) strains from children, dogs, and chickens. Background colors for each clonal relationship match legend in Figure 2. Longer distances are indicated by a lighter color font; distance of 0 km indicates samples were collected from the same household. Note: Asterisk indicates individuals who shared isolates in multiple CRs.

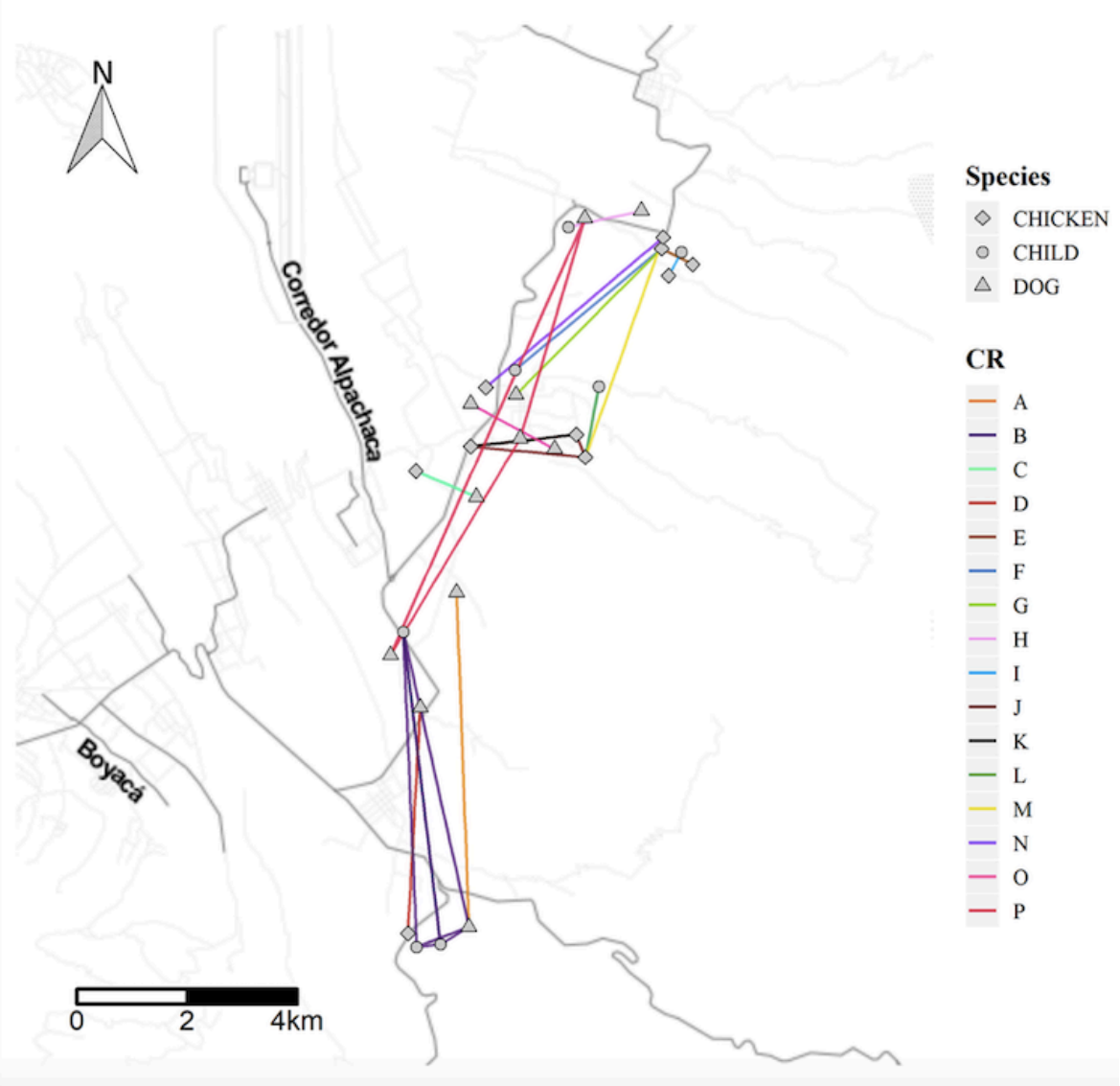

Figure 2. Map of clonal relationships (CRs) among third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) strains in children, dogs, and chickens in peri-urban study site east of Quito, Ecuador.

Genotypes of 3GCR-EC Isolates

We constructed a maximum likelihood tree based on the core genomes to compare the phylogeny of isolates associated with their origin. The genomes of *E. coli* isolates from children, dogs, and chickens were intermixed and distributed across the phylogeny, with little evidence of clustering by host animal species (Figure 3). When isolates were characterized by Clermont phylogenetic typing, most isolates belonged to phylogroup A, which accounted for 33.7% (*n* = 89) of total isolates. In this phylogroup, we identified *E. coli* from children (*n* = 28),

dogs (*n* = 34) and chickens (*n* = 27). Phylogroup B1 accounted for 25% (*n* = 66) of isolates; from children (*n* = 9), from dogs (*n* = 37), and from chickens (*n* = 20). Phylogroups D, F, E, and C accounted for 15.9% (*n* = 42), 10.6% (*n* = 28), 10.2% (*n* = 27), and 4.5% (*n* = 12) of isolates, respectively. All phylogroups were represented by isolates from children, dogs, and chickens (Figure 3, Figure S1). MLST analysis based on 7 housekeeping genes showed that 252 isolates were assigned to 44 known STs, whereas 12 isolates represented 8 novel STs. Seven STs were shared by 44.3% (*n* = 117) of isolates from all three sources: ST38 (children = 20, dogs = 1, chickens = 2), ST10 (children = 9, dogs = 2, chickens = 8), ST117 (children = 8, dogs = 5, chickens = 6), ST2847 (children = 5, dogs = 1, chickens = 11), ST155 (children = 1, dogs = 7, chickens = 7), ST58 (children = 5, dogs = 7, chickens = 1) and ST48 (children = 5, dogs = 2, chickens = 4). In contrast, 35 STs were only observed in isolates from one source type: children (8 STs; *n* = 22), dogs (15 STs; *n* = 41) or chickens (12 STs; *n* = 23). The application of a cgMLST scheme showed 86 STs, of which only 2, ST80776 (children = 5, dogs = 1, chickens = 10) and ST40001 (children = 1, dogs = 1, chickens = 2), were assigned to isolates from all three sources. Several isolates belonging to the same ST based on 7 genes were assigned to different STs based on cgMLST (Figure 3). Additionally, we identified 74 different serotypes in 264 isolates, of which only 4 were represented by isolates across all three species. Serotype O8:H25 accounted for 4.9% (*n* = 13) of isolates (children = 5, dogs = 7, chickens = 1). Serotype O8:H9 accounted for 4.5% (*n* = 12) of isolates (children = 5, dogs = 5, chickens = 2). Serotype O89:H10 accounted for 3.4% (*n* = 9) of isolates (children = 1, dogs = 6, chickens = 2). Serotype O109:H9 accounted for 1.5% (*n* = 4) of isolates (children = 1, dogs = 1, chickens = 1). Serotypes and MLST profiles of all isolates are shown in Excel Table S2.

Antimicrobial Susceptibility and bla$_{CTX-M}$ Gene Detection in 3GCR-EC Isolates

Most 3GCR-EC, 175 (66.3%) of 264 isolates, were resistant to between five and seven antimicrobial drugs (range = 3–10; median = 6) (Figure 3), but 3 isolates (two from chickens and one from a dog) were resistant to 10 of 12 antimicrobials evaluated. Presence of AMR genes in the whole genome sequences of the 264 *E. coli* isolates, investigated by ResFinder, showed numerous ESBL-encoding *bla*$_{CTX-M}$ gene variants were distributed in isolates from humans and domestic animals (Figure 3 and Figure 4). Among the 264 3GCR-EC isolates, we identified allelic variants of *bla*$_{CTX-M}$ in 224 (84.5%). The most common allelic variant was *bla*$_{CTX-M-55}$ in 69 isolates (30.8%), found in similar proportions in isolates from children (n = 22), dogs (n = 20), and chickens (n = 27); $\chi^2(5, n = 224) = 5.6346$, $p = 0.060$. The second most common allele was *bla*$_{CTX-M-65}$ in 56 isolates (25%), more commonly identified in dog isolates (n = 34) rather than chicken (n = 15) and child (n = 7) isolates; $\chi^2(5, n = 224) = 23.5066$, $p < 0.00001$ (Figure 4). In several of the CRs identified, we found different phenotypic AMR profiles (13 CRs), AMR genes (14 CRs), and plasmid replicons (15 CRs) within members of the same CR (Tables S18-S33).

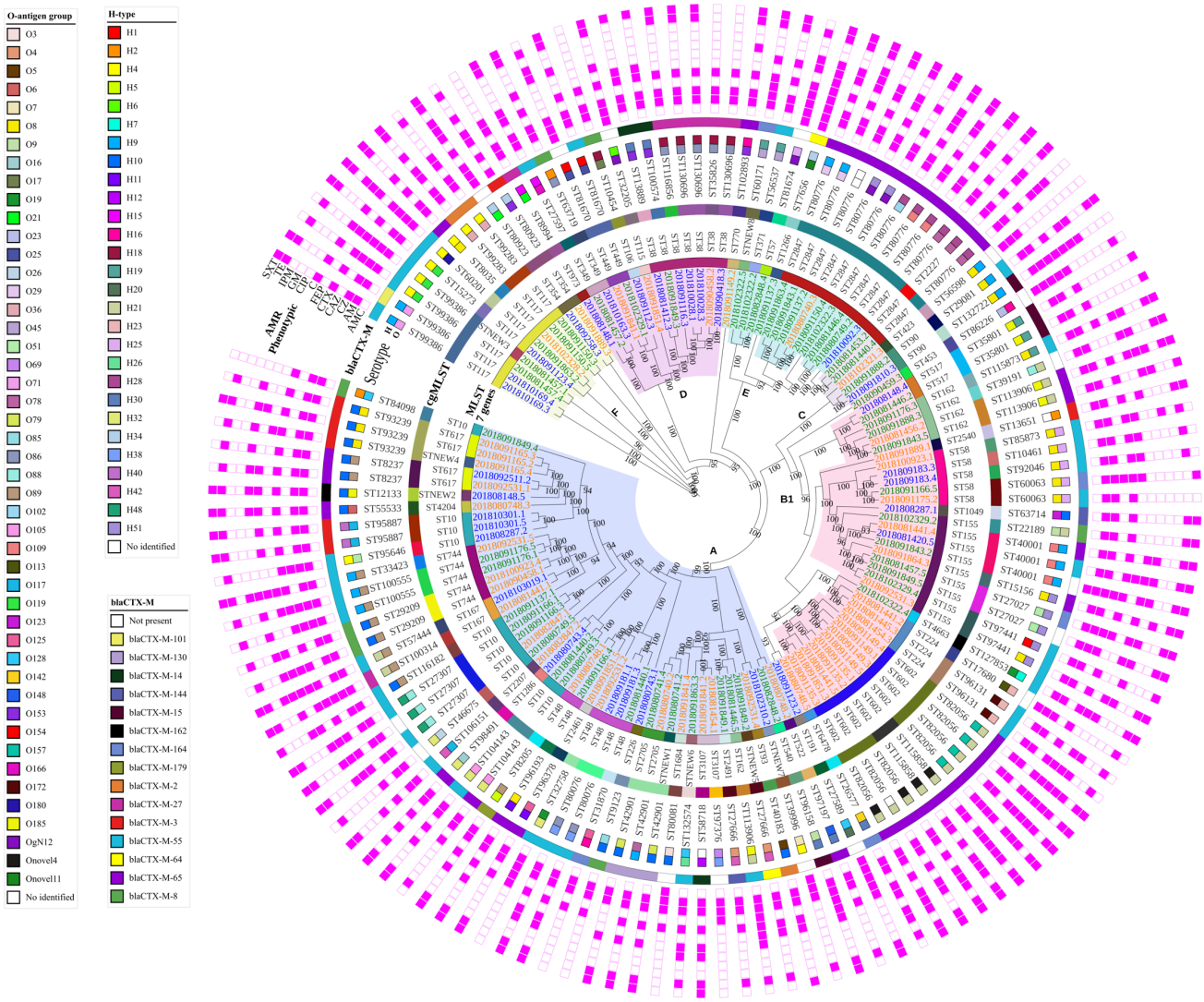

Figure 3. Maximum-likelihood phylogenetic tree of 131 third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolates from children, dogs, and chickens based on core genomes. Labels show isolate ID assigned based on host ID followed by its isolate number. Origin of isolate is shown by font colors (child: blue; dog: orange; chicken: green). Background colors indicate the six phylogroups identified. Sequences types (STs) based on multilocus sequence typing (MLST) of seven housekeeping genes are shown in the color-coded inner ring. STs based on core genome MLST (cgMLST) are shown in the color-coded middle ring. Predicted serotypes are shown with combination of colored squares for (O-antigen group and H-type). The color-coded outer ring represents the allelic variant of blaCTX-M. Pink-colored squares indicate resistance to different antimicrobials. Note: AMC, amoxicillin-clavulanate; AM, ampicillin; CZ, cefa-zolin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; C, chloramphenicol; CIP, ciprofloxacin; GM, gentamicin; IPM, imipenem; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole.

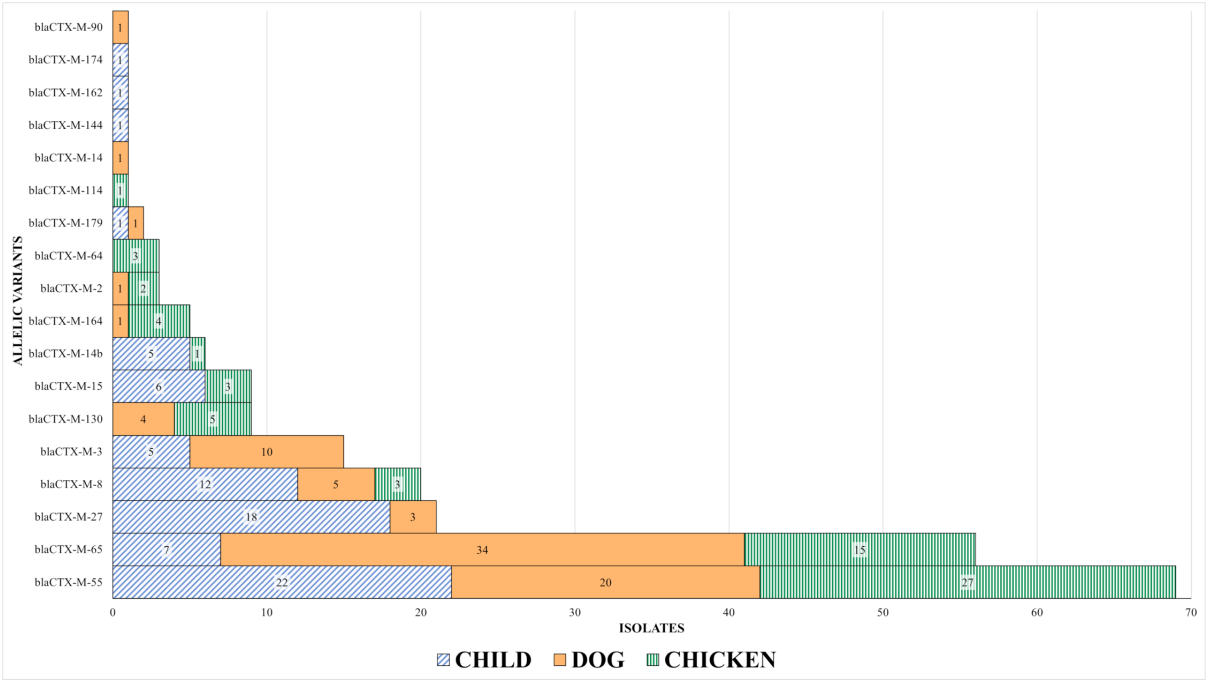

Figure 4. Frequency of allelic variants of *bla*$_{CTX-M}$ in third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolates from children (blue diagonal lines), dogs (orange), and chickens (green vertical lines).

Discussion

We found 16 CRs of 3GCR-EC isolates shared by different domestic animals and children in semi-rural communities of Ecuador using a pairwise SNPs analysis in the core genome sequences. Half of the CRs were shared by members of the same animal species and the other half were shared among different animal species (Figure 2). Also, the same allelic variants of *bla*$_{CTX-M}$ were found in domestic animals and children (Figure 3 and Figure 4). The presence of isolates with CRs and the same allelic variants of *bla*$_{CTX-M}$ in children and domestic animals indicates a shared population of *E. coli* among different host species. This finding suggests that many strains of *E. coli* can efficiently colonize the intestines of different animal species. This is in striking contrast with recent reports (from Europe) which concluded that the population of ESBL-producing *E. coli* and allelic variants of *bla*$_{CTX-M}$ from humans were different from those present in domestic animals or animal products (Day et al. 2019; de Been et al. 2014; Ludden

et al. 2019). We hypothesize that spatiotemporal differences in which other researchers have collected isolates (Day et al. 2019; de Been et al. 2014; Ludden et al. 2019), which was not the case for this study, could be one of the reasons for the lack of relatedness among human and other animal isolates due to rapid turnover and high diversity of *E. coli* strains that circulate simultaneously in human communities (Richter et al. 2018; Salinas et al. 2019). The genetic similarity of strains among domestic animals and humans is a strong evidence that many *E. coli* lineages are generalists and able to colonize the intestines of different animal species. This is consistent with the identification of the same phylogroups and STs among isolates from children, dogs and chickens (Figure 3). The high diversity of serotypes identified in this study may have been due to the fact that the O-antigen is subject to strong selection pressure from the immune system and also from predation by bacteriophages (Ingle et al. 2016).

This study provides strong evidence for overlap of commensal *E. coli* strains and AMR genes within different species, which could be indicative of probable movement among humans and domestic animals in the same community across relatively large distances (i.e., not just in the surrounding household environment). The design of this study, which matched children's and domestic animals' sample collections in space and time, allowed us to draw different conclusions about the relationship of *E. coli* populations in comparison with past studies that have suggested that these populations of *E. coli* are unrelated. We observed free-ranging chickens and dogs in the household outdoor environment, which may increase the likelihood of direct and frequent contact with children (Table 3), considered as a risk factor of AMR transmission (Li et al. 2019; Pomba et al. 2017). In addition, in most of the study households, domestic animal feces deposited in the household environment are often stored to be used as an organic fertilizer (Table 2). This close relationship among humans and

domestic animals has also been described in LMICs, as well as rural areas of upper-middle-income countries (UMICs) where genetically related *E. coli* strains were shared between humans and domestic animals (Borges et al. 2019; Li et al. 2019); however, our study is the second showing conclusive evidence from WGS and shows a larger number of genetically related isolates in domestic animals and humans (Li et al. 2019). Human exposure to animal feces in rural households has been considered potentially hazardous for zoonotic transmission of enteropathogens in LMICs, despite having improved WaSH conditions (Prendergast et al. 2019). It is important to note that the households in this study had toilet facilities connected to sewer lines or septic tanks, children's feces were safely disposed of, and most of households had handwashing facilities with water and soap available. The households' main source of drinking water was piped water inside the home, and in several cases, additional water treatment was reported prior to consumption (Table 1). In this context, our findings suggest that fecal contamination of the household environment by domestic animals likely plays an important role in the transmission of AMR in the community; however, we acknowledge a limitation of this study; we failed to determine the transmission directionality (human-to-animal or animal-to-human transmission). There could be other routes of exposure to AMR, which we did not explore here, such as untreated wastewater that is released to rivers and other waterways in Ecuador (Ortega-Paredes et al. 2020). Furthermore, this area is marked by large-scale poultry production operations, which could be an important source of AMR in this community.

Most CRs showed different phenotypic AMR profiles, AMR genes, and plasmid replicons within members of the same CR. Therefore, these findings are evidence of highly

dynamic horizontal transfer of AMR genes and mobile genetic elements (MGEs) in the *E. coli* community.

Half of all pairs of CR samples were from households between 2.7 and 9 km apart, and 22 of 25 pairs were not from the same household (Figure 1). Most studies for risk factors for AMR have focused on individual-level or household-level risk factors. The spread of clonally related resistant *E. coli* over significant distances in our study area suggests that community-level factors may be driving the spread of resistance. In contrast, the presence of backyard chickens in a community in Peru was associated with decreased prevalence of multidrug-resistant *E. coli* among children (Kalter et al. 2010). An exploratory study determined that both backyard and commercial poultry production are prevalent in the area of our study, and antimicrobials are commonly used for growth promotion and disease prevention (Lowenstein et al. 2016). Poultry production may be one of many important community-level drivers of antimicrobial resistance transmission. Additional research is needed to compare the relative importance of individual- vs. community-level drivers of antimicrobial resistance to inform the most effective and appropriate intervention strategies. Another limitation is that each isolate was sequenced only once, and this limited our ability to measure between-run precision and include WGS reproducibility controls.

This study provides evidence that domestic animals play an important role spreading ESBL resistance to the microbiota of young children. We also show evidence that the environment–contaminated by domestic animal feces–serves as a potentially important source of clinically relevant antimicrobial-resistant bacteria and AMR genes that likely move with high frequency among domestic animals and young children. Furthermore, the spread of AMR occurs beyond the household environment and extends across relatively large distances

in the community. Our study adds to the body of evidence indicating that control of antimicrobial resistance in human clinical medicine must include reduction of antimicrobial resistance in domestic animals.

Acknowledgments

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Supplementary Material

Table S1. Joint distribution of the number of dogs and chickens owned per household

	No. dogs owned						
No. chickens owned	0	1	2	3	9	12	Total
0	10	0	0	0	0	0	10
1-5	0	1	6	1	1	0	9
6-10	0	0	3	1	0	0	4
11-25	0	1	2	1	0	1	5
>25	0	0	1	0	0	0	1
Total	10	2	12	3	1	1	29

Table S2. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship A

		2018100923 Dog	
		isolate2	isolate4
2018090458 Dog	isolate1	7	11
	isolate3	1	5
	isolate5	0	4

Table S3. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship B

		2018090458 Dog[a]		**2018090418 Child[a]**					**2018091116 Child**					**201810028 Child[b]**			
		isolate 2	isolate 4	isolate 1	isolate 2	isolate 3	isolate 4	isolate 5	isolate 1	isolate 2	isolate 3	isolate 4	isolate 5	isolate 2	isolate 3	isolate 4	isolate 5
2018090458 Dog[a]	isolate2	------		0	0	0	15	5	63	38	71	52	52	29	40	29	39
	isolate4		------	3	3	3	18	8	66	41	74	55	55	32	43	32	42
2018090418 Child[a]	isolate1	0	3	------					63	38	71	52	52	29	40	29	39
	isolate2	0	3		------				63	38	71	52	52	29	40	29	39
	isolate3	0	3			------			63	38	71	52	52	29	40	29	39
	isolate4	15	18				------		78	53	83	67	66	44	55	44	54
	isolate5	5	8					------	68	43	76	57	57	34	45	34	44
2018091116 Child	isolate1	63	66	63	63	63	78	68	------					59	70	59	69
	isolate2	38	41	38	38	38	53	43		------				35	46	35	45
	isolate3	71	74	71	71	71	83	76			------			72	83	72	82
	isolate4	52	55	52	52	52	67	57				------		50	61	50	60
	isolate5	52	55	52	52	52	66	57					------	51	61	51	61
201810028 Child[b]	isolate2	29	32	29	29	29	44	34	59	35	72	50	51	------			
	isolate3	40	43	40	40	40	55	45	70	46	83	61	61		------		
	isolate4	29	32	29	29	29	44	34	59	35	72	50	51			------	
	isolate5	39	42	39	39	39	54	44	69	45	82	60	61				------

[a]Individuals that share same household.
[b]Child does not live with domestic animals

Table S4. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship C

		2018080741 Chicken[a]			
		isolate1	isolate3	isolate4	isolate5
2018080740 Dog[a]	isolate2	57	39	36	29
	isolate4	53	33	30	23
	isolate5	52	31	28	22

[a]Individuals that share same household.

Table S5. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship D

		2018091166 Chicken
		isolate1
2018082847 Dog	isolate1	44
	isolate4	44

Table S6. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship E

		2018091863 Chicken
		isolate4
2018091843 Chicken	isolate1	70
	isolate3	34

Table S7. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship F

		2018091843 Chicken	
		isolate2	isolate4
2018081420 Child	isolate5	38	32

Table S8. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship G

		2018091843 Chicken
		isolate5
2018081456 Dog	isolate1	79
	isolate2	60
	isolate3	49
	isolate5	54

Table S9. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship H

		2018092531 Dog
		isolate1
2018092511 Child[a]	isolate2	40

[a]Child does not live with domestic animals.

Table S10. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship I

		2018091888 Chicken	
		isolate1	isolate2
2018091810 Child	isolate1	88	92
	isolate2	74	78
	isolate4	61	65
	isolate5	72	76

Table S11. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship J

		2018081446 Chicken	**2018080749 Chicken**		**2018081440 Chicken**
		isolate4	isolate1	isolate2	isolate4
2018081446 Chicken	isolate4	------	32	44	85
2018080749 Chicken	isolate1	32	------		114[a]
	isolate2	44		------	126[a]
2018081440 Chicken	isolate4	85	114[a]	126[a]	------

[a]Values were not considered part of the clonal relationship J.

Table S12. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship K

		2018081440 Chicken
		isolate2
2018080749 Chicken	isolate3	71

Table S13. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship L

		2018081446 Chicken[a]
		isolate2
201808148 Child[a]	isolate4	87

[a]Individuals that share same household.

Table S14. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship M

		2018091849 Chicken
		isolate1
2018081446 Chicken	isolate3	70
	isolate5	59

Table S15. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship N

		2018091849 Chicken
		isolate5
2018081457 Chicken	isolate5	37

Table S16. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship O

		2018081454 Dog
		isolate3
2018081445 Dog	isolate2	27
	isolate3	24
	isolate5	35

Table S17. Number of single nucleotide polymorphisms (SNPs) for each pairwise analysis between strains from clonal relationship P

		2018081441 Dog	**2018091851 Dog**	**2018091135 Dog**		
		isolate5	isolate3	isolate 3	isolate 4	isolate 5
2018081441 Dog	isolate 5	------	49	90	86	80
2018091851 Dog	isolate 3	49	------	64	83	79
2018091135 Dog	isolate 3	90	64	------		
	isolate 4	86	83		------	
	isolate 5	80	79			------

Table S18. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship A

ISOLATE	**PHENOTYPIC AMR**	**AMR GENES**	**PLASMID REPLICONS**
2018090458isolate1 Dog	CZ-CIP-AM-C-FEP-CTX-TE	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, bla*$_{CTX\text{-}M\text{-}8}$*, catA1, mdf(A), sul2, tet(B)*	Col(MG828), IncFIA, IncFIB(AP001918), IncI1, IncQ1
2018090458isolate3 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX\text{-}M\text{-}8}$*, catA1, mdf(A), sul2, tet(B)*	IncFIA, IncFIB(AP001918), IncI1, IncQ1
2018090458isolate5 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX\text{-}M\text{-}8}$*, catA1, mdf(A), sul2, tet(B)*	IncFIA, IncFIB(AP001918), IncI1, IncQ1
2018100923isolate2 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX\text{-}M\text{-}8}$*, catA1, mdf(A), sul2, tet(B)*	IncFIA, IncFIB(AP001918), IncI1, IncQ1
2018100923isolate4 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX\text{-}M\text{-}8}$*, catA1, mdf(A), sul2, tet(B)*	IncFIA, IncFIB(AP001918), IncI1, IncQ1

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; FEP: cefepime; CTX: cefotaxime; TE: tetracycline.

Table S19. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship B

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018090458isolate2 Dog[a]	CZ-AM-CTX	*bla*$_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090458isolate4 Dog[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090418isolate1 Child[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090418isolate2 Child[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090418isolate3 Child[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090418isolate4 Child[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018090418isolate5 Child[a]	CZ-AM-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018091116isolate4 Child	CZ-AM-FEP-CTX	*bla* $_{CTX-M-27}$, *mdf(A)*	IncFIB(AP001918), IncFII
2018091116isolate1 Child	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII
2018091116isolate2 Child	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII
2018091116isolate3 Child	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII
2018091116isolate5 Child	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII
201810028isolate2 Child[b]	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII, IncI1
201810028isolate3 Child[b]	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII, IncI1
201810028isolate4 Child[b]	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII, IncI1
201810028isolate5 Child[b]	CZ-AM-SXT-CTX-TE	*aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-27}$, *dfrA17, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFII, IncI1

Note: CZ: cefazolin; AM: ampicillin; CTX: cefotaxime; FEP: cefepime; SXT: trimethoprim-sulfamethoxazole; TE: tetracycline.
[a]Individuals that share same household. [b]Child does not live with domestic animals.

Table S20. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship C

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018080740isolate2 Dog[a]	CZ-AM-C-SXT-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-130}$*, blaTEM-1B, dfrA14, floR, mdf(A), qnrS1, sul2, tet(A)*	p0111
2018080740isolate4 Dog[a]	CZ-AM-C-SXT-CTX-TE	*aph(6)-Id, bla* $_{CTX-M-130}$*, bla*$_{TEM-1B}$*, dfrA14, floR, mdf(A), tet(A)*	p0111
2018080740isolate5 Dog[a]	CZ-AM-C-SXT-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-130}$*, bla*$_{TEM-1B}$*, dfrA14, floR, mdf(A), qnrS1, sul2, tet(A)*	p0111
2018080741isolate1 Chicken[a]	CZ-AM-C-SXT-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-130}$*, bla*$_{TEM-1B}$*, dfrA14, floR, mdf(A), qnrS1, sul2, tet(A)*	p0111
2018080741isolate3 Chicken[a]	CZ-AM-C-SXT-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-130}$*, bla*$_{TEM-1B}$*, dfrA14, floR, mdf(A), qnrS1, sul2, tet(A)*	p0111
2018080741isolate4 Chicken[a]	CZ-AM-C-SXT-CTX-TE	*bla* $_{CTX-M-130}$*, dfrA14, floR, mdf(A), qnrS1, tet(A)*	-----
2018080741isolate5 Chicken[a]	CZ-AM-C-SXT-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-130}$*, bla*$_{TEM-1B}$*, dfrA14, floR, mdf(A), qnrS1, sul2, tet(A)*	p0111

Note: CZ: cefazolin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; CTX: cefotaxime; TE: tetracycline.
[a]Individuals that share same household.

Table S21. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship D

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018082847isolate1 Dog	CZ-AM-C-SXT-FEP-CTX-TE	*aadA1, bla*$_{CTX-M-55}$*, dfrA1, floR, fosA3, mdf(A), qnrB19, sul3, tet(A)*	IncFII(pHN7A8), IncI1, IncN, IncX1
2018082847isolate4 Dog	CZ-AM-C-SXT-FEP-CTX-TE	*aadA1, bla* $_{CTX-M-55}$*, bla*$_{TEM-141}$*, dfrA1, floR, fosA3, mdf(A), qnrB19, sul3, tet(A)*	IncFII(pHN7A8), IncI1, IncN, IncX1
2018091166isolate1 Chicken	CZ-AM-C-SXT-FEP-CTX-TE	*aadA1, bla* $_{CTX-M-55}$*, bla*$_{TEM-141}$*, dfrA1, floR, fosA3, mdf(A), qnrB19, sul3, tet(A)*	Col156, IncFII(pHN7A8), IncN, IncX1

Note: CZ: cefazolin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; FEP: cefepime; CTX: cefotaxime; TE: tetracycline.

Table S22. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship E

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018091843isolate1 Chicken	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IVa, aadA1, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, $bla_{CTX-M-65}$, dfrA17, floR, fosA3, mdf(A), sul2, tet(A)*	IncFIB(AP001918)
2018091843isolate3 Chicken	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IVa, aadA1, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, $bla_{CTX-M-65}$, dfrA17, floR, fosA3, mdf(A), sul2, tet(A)*	IncFIB(AP001918)
2018091863isolate4 Chicken	CZ-CIP-AM-C-SXT-CTX-TE	*aac(3)-IVa, aadA1, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, $bla_{CTX-M-65}$, dfrA17, floR, fosA3, mdf(A), sul2, tet(A)*	IncFIB(AP001918), p0111

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; GM: gentamicin; CTX: cefotaxime; TE: tetracycline.

Table S23. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship F

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081420isolate5 Child	CZ-CIP-AM-C-SXT-FEP-CTX-TE	*aadA1, aadA2, aph(3'')-Ib, aph(6)-Id, $bla_{CTX-M-55}$, blaTEM-141, cmlA1, floR, mdf(A), sul2, sul3, tet(A)*	IncFIB(AP001918), IncFII(pCoo)
2018091843isolate2 Chicken	CZ-CIP-AM-C-SXT-CAZ-CTX-TE-AMC	*aadA1, aadA2, bla_{CMY-2}, cmlA1, dfrA12, mdf(A), sul3, tet(A)*	IncFIB(AP001918), IncFII(pCoo), IncI1, IncI2
2018091843isolate4 Chicken	CZ-CIP-AM-C-SXT-CAZ-CTX-TE-AMC	*aadA1, aadA2, bla_{CMY-2}, cmlA1, dfrA12, mdf(A), sul3, tet(A)*	IncFIB(AP001918), IncFII(pCoo), IncI1, IncI2

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; FEP: cefepime; CTX: cefotaxime; TE: tetracycline; AMC: amoxicillin-clavulanate; CAZ: ceftazidime.

Table S24. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship G

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081456isolate1 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IId, aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-65}$*,* *bla* $_{TEM-1B}$*, dfrA17, floR, fosA3, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncN
2018081456isolate2 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IId, aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-65}$*,* *bla* $_{TEM-1B}$*, dfrA17, floR, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncN
2018081456isolate3 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IId, aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-65}$*,* *bla* $_{TEM-1B}$*, dfrA17, floR, fosA3, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncN
2018081456isolate5 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IId, aadA5, aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-65}$*,* *bla* $_{TEM-1B}$*, dfrA17, floR, fosA3, mdf(A), mph(A), sul1, sul2, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncN
2018091843isolate5 Chicken	CZ-CIP-AM-SXT-GM-CTX	*aac(3)-IId, aadA5, bla* $_{CTX-M-65}$*, dfrA17, fosA3, mdf(A), mph(A), sul2*	IncFIB(AP001918), IncFIC(FII)

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; GM: gentamicin; CTX: cefotaxime; TE: tetracycline.

Table S25. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship H

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018092511isolate2 Child[a]	CZ-CIP-AM-CTX-TE	*bla* $_{CTX-M-65}$*, fosA3, mdf(A), tet(B)*	IncFIA, IncFIB(AP001918), IncFIC(FII), IncI1
2018092531isolate1 Dog	CZ-CIP-AM-C-CTX-TE	*aadA1, aph(3')-Ia, bla* $_{CTX-M-65}$*, floR, fosA3, mdf(A), qnrB19, sul3, tet(A), tet(B)*	IncFIA, IncFIB(AP001918), IncFIC(FII), IncI1

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; CTX: cefotaxime; TE: tetracycline; C: chloramphenicol.
[a]Child does not live with domestic animals.

Table S26. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship I

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018091810isolate1 Child	CZ-CIP-AM-C-CAZ-CTX	*aadA1, aadA2, bla*$_{CTX-M-15}$*, cmlA1, mdf(A), qnrB19, sul3*	IncFIB(AP001918), IncFIC(FII)
2018091810isolate2 Child	CZ-CIP-AM-C-CAZ-FEP-CTX	*aadA1, aadA2, bla* $_{CTX-M-15}$*, cmlA1, mdf(A), qnrB19, sul3*	IncFIB(AP001918), IncFIC(FII)
2018091810isolate4 Child	CZ-CIP-AM-C-CAZ-FEP-CTX	*aadA1, aadA2, bla* $_{CTX-M-15}$*, cmlA1, mdf(A), qnrB19, sul3*	IncFIB(AP001918), IncFIC(FII)
2018091810isolate5 Child	CZ-CIP-AM-C-CAZ-FEP-CTX	*aadA1, aadA2, bla* $_{CTX-M-15}$*, cmlA1, mdf(A), qnrB19, sul3*	IncFIB(AP001918), IncFIC(FII)
2018091888isolate1 Chicken	CZ-CIP-AM-C-SXT-CAZ-CTX-TE	*aadA1, bla* $_{CTX-M-55}$*, bla*$_{TEM-141}$*, dfrA1, floR, fosA3, mdf(A), sul3, tet(A)*	IncFIA, IncFIB(AP001918), IncFII(pHN7A8), IncI1, IncN
2018091888isolate2 Chicken	CZ-CIP-AM-C-SXT-CTX-TE	*aadA1, bla* $_{CTX-M-55}$*, bla*$_{TEM-141}$*, dfrA1, floR, fosA3, mdf(A), sul3, tet(A)*	IncFIA, IncFIB(AP001918), IncFII(pHN7A8), IncI1, IncN

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; CAZ: ceftazidime; CTX: cefotaxime; FEP: cefepime; SXT: trimethoprim-sulfamethoxazole; TE: tetracycline.

Table S27. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship J

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081446isolate4 Chicken	CZ-CIP-AM-SXT-GM-CTX-TE	*aac(3)-IVa, aadA1, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla* $_{CTX-M-65}$*, dfrA17, mdf(A), sul2, tet(A)*	IncFIB(AP001918)
2018080749isolate1 Chicken	CZ-CIP-AM-C-SXT-CTX-TE	*aadA1, aac(3)-IVa, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla* $_{CTX-M-65}$*, dfrA17, floR, mdf(A), sul2, tet(A)*	Col(MG828), IncFIB(AP001918)
2018080749isolate2 Chicken	CZ-CIP-AM-C-SXT-CTX-TE	*aadA1, aac(3)-IVa, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id,* *bla*$_{CTX-M-65}$*, dfrA17, floR, mdf(A), sul2, tet(A)*	IncFIB(AP001918)
2018081440isolate4 Chicken	CZ-CIP-AM-C-GM-CTX	*aac(3)-IVa, aadA1, aph(4)-Ia,* $_{blaCTX-M-65}$*, floR, fosA3, mdf(A)*	ColpVC, IncI2

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; SXT: trimethoprim-sulfamethoxazole; GM: gentamicin; CTX: cefotaxime; TE: tetracycline; C: chloramphenicol.

Table S28. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship K

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018080749isolate3 Chicken	CZ-AM-CTX-TE	*bla*$_{CTX-M-65}$*, fosA3, mdf(A), qnrS1, tet(A)*	IncFII(pHN7A8), Incl1, IncY
2018081440isolate2 Chicken	CZ-AM-C-SXT-CTX-TE	*aadA1, aadA2, bla*$_{CTX-M-55}$*, bla*$_{TEM-141}$*, dfrA1, floR, mdf(A), qnrB19, sul3, tet(A)*	IncFII(pHN7A8), IncX1, IncY

Note: CZ: cefazolin; AM: ampicillin; CTX: cefotaxime; TE: tetracycline; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole.

Table S29. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship L

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
201808148isolate4 Child[a]	CZ-AM-SXT-CTX	*aadA5, bla* $_{CTX-M-15}$*, dfrA17, mdf(A), mph(A), qnrB19, qnrS1, sul1*	IncB/O/K/Z, IncFII
2018081446isolate2 Chicken[a]	CZ-AM-SXT-CTX	*aadA5, bla*$_{CTX-M-15}$*, dfrA17, mdf(A), mph(A), qnrS1, sul1*	Col156, IncB/O/K/Z, IncFIB(AP001918), IncFII

Note: CZ: cefazolin; AM: ampicillin; SXT: trimethoprim-sulfamethoxazole; CTX: cefotaxime.
[a]Individuals that share same household.

Table S30. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship M

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081446isolate3 Chicken	CZ-AM-C-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla*$_{CTX-M-55}$*, floR, mdf(A), qnrB19, sul2*	-----
2018081446isolate5 Chicken	CZ-AM-C-CAZ-FEP-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla*$_{CTX-M-55}$*, bla*$_{TEM-141}$*, floR, mdf(A), qnrB19, sul2, tet(A)*	IncFII(pHN7A8)
2018091849isolate1 Chicken	CZ-AM-CTX-TE	*bla*$_{TEM-141}$*, fosA3, mdf(A), tet(A)*	IncFIB(AP001918), IncR

Note: CZ: cefazolin; AM: ampicillin; C: chloramphenicol; CTX: cefotaxime; TE: tetracycline; CAZ: ceftazidime; FEP: cefepime.

Table S31. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship N

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081457isolate5 Chicken	CZ-CIP-AM-C-SXT-CAZ-CTX-TE	*aadA2, cmlA1, mdf(A), sul3, tet(A)*	IncFIB(AP001918), IncFII(pHN7A8)
2018091849isolate5 Chicken	CZ-CIP-AM-C-SXT-CAZ-TE	*aadA1, aadA2, bla*$_{SHV-12}$*, cmlA, dfrA12, mdf(A), mef(B), sul3, tet(A)*	IncFIB(AP00198), IncFII(pCoo)

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; CAZ: ceftazidime; CTX: cefotaxime; TE: tetracycline.

Table S32. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship O

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081445isolate2 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla* $_{CTX-M-55}$*, bla*$_{TEM-141}$*, floR, mdf(A), sul2, tet(A)*	IncFIB(AP001918), IncFII(pHN7A8), IncI2, IncN
2018081445isolate3 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla*$_{CTX-M-55}$*, bla*$_{TEM-141}$*, floR, mdf(A), sul2, tet(A)*	IncFIB(AP001918), IncFII(pHN7A8), IncI2, IncN
2018081445isolate5 Dog	CZ-CIP-AM-C-CTX-TE	*aph(3'')-Ib, aph(6)-Id, bla*$_{CTX-M-55}$*, floR, mdf(A), sul2, tet(A)*	IncFIB(AP001918), IncFII(pHN7A8), IncI2, IncN
2018081454isolate3 Dog	CZ-CIP-AM-C-FEP-CTX-TE	*aadA1, aadA2, aph(3'')-Ib, aph(6)-Id, bla*$_{CTX-M55}$*, bla*$_{TEM-1B}$*, cmlA1, floR, mdf(A), sul2, sul3, tet(A)*	IncFIB(AP001918), IncFII(pHN7A8), IncX1, IncX4

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; CTX: cefotaxime; TE: tetracycline; FEP: cefepime.

Table S33. Phenotypic antimicrobial resistance (AMR), AMR genes and plasmid replicons profiles for each strain from clonal relationship P

ISOLATE	PHENOTYPIC AMR	AMR GENES	PLASMID REPLICONS
2018081441isolate5 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IVa, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *catA1, dfrA17, floR, fosA3, fosA7, mdf(A), sul2, tet(B)*	IncFIB(AP001918), IncFIC(FII), IncI1, IncQ1, p0111
2018091851isolate3 Dog	CZ-CIP-AM-C-SXT-CTX-TE	*aac(3)-IVa, aadA1, aadA2, aph(4)-Ia, bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *cmlA1, dfrA12, floR, fosA3, fosA7, mdf(A), sul3, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncI1
2018091135isolate3 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aadA1, aadA2, aac(3)-IVa, aph(3'')- IIa, aph(4)-Ia, bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *cmlA1, dfrA12, floR, fosA3, fosA7, mdf(A), sul3, tet(A)*	IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncI1, IncN, IncX1
2018091135isolate4 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IVa, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *catA1, dfrA17, floR, fosA3, fosA7, mdf(A), sul2, tet(B)*	IncFIB(AP001918), IncFIC(FII), IncI1, IncQ1
2018091135isolate5 Dog	CZ-CIP-AM-C-SXT-GM-CTX-TE	*aac(3)-IVa, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla*$_{CTX-M-65}$, *bla*$_{TEM-1B}$, *catA1, dfrA17, floR, fosA3, fosA7, mdf(A), sul2, tet(B)*	IncFIB(AP001918), IncFIC(FII), IncI1, IncQ1

Note: CZ: cefazolin; CIP: ciprofloxacin; AM: ampicillin; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole; GM: gentamicin; CTX: cefotaxime; TE: tetracycline.

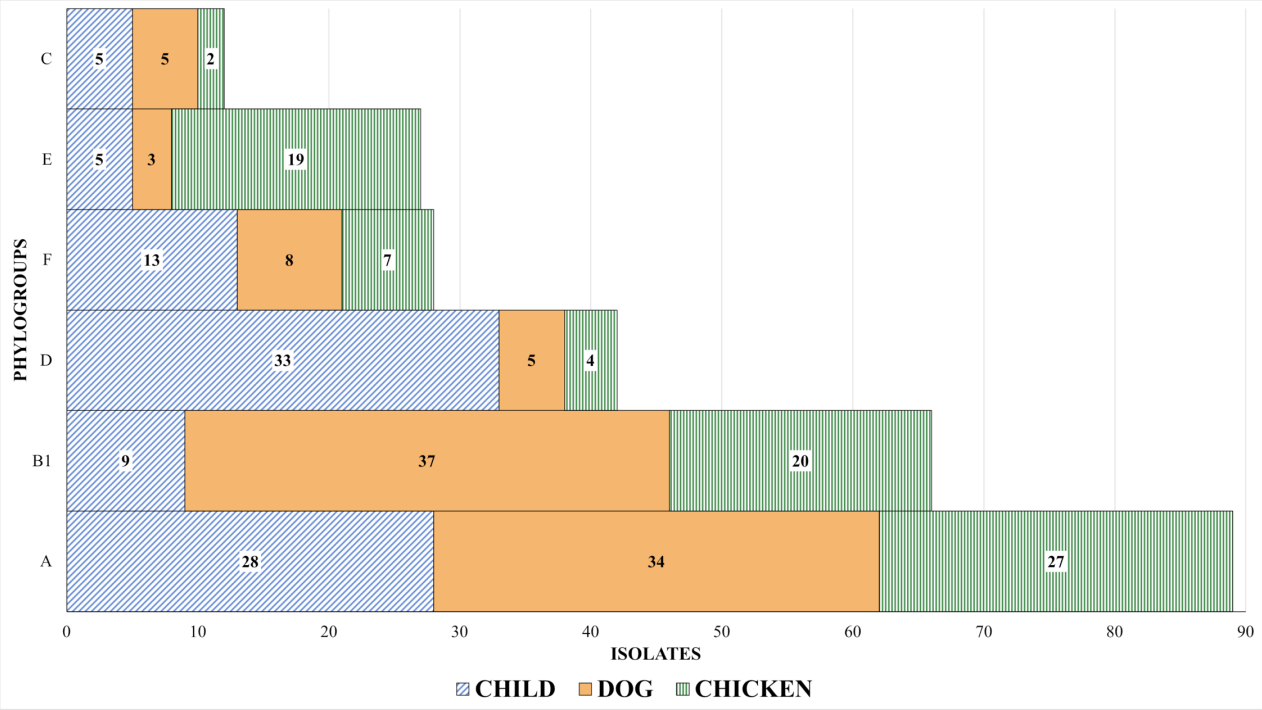

Figure S1. Frequency of phylogroups in third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolates from Children (blue diagonal lines), Dogs (orange), and Chickens (green vertical lines).

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CHAPTER 3

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IS*26* drives the dissemination of *bla*$_{CTX-M}$ genes in an Ecuadorian community

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ABSTRACT The rapid dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, mainly *Escherichia coli* carrying *bla*$_{CTX-M}$ genes, is a major public health concern due to its successful spread in hospital settings as well as among humans and animals in the community. We characterized ESBL-producing *E. coli* isolates from children and domestic animals in semirural communities of Ecuador to assess the contribution of horizontal gene transfer of the *bla*$_{CTX-M}$ genes among *E. coli* isolates.

From 20 selected *E. coli* isolates (from children and domestic animals) harboring *bla*$_{CTX-M}$ allelic variants, we identified 16 plasmids carrying *bla*$_{CTX-M-55}$ (n = 9), *bla*$_{CTX-M-65}$ (n = 5), *bla*$_{CTX-M-27}$ (n = 2), as well as four *bla*$_{CTX-M-65}$ carried on chromosomes. The backbone structure of plasmids including replication, maintenance, and plasmid transfer genes and the synteny were conserved in all plasmids carrying the same *bla*$_{CTX-M}$ allelic variant. In all plasmids and chromosomes, the *bla*$_{CTX-M}$ genes were bracketed by two IS*26* transposable elements. This study highlights the critical role of the IS*26* transposable element for the current mobility of *bla*$_{CTX-M}$ genes among plasmids or from plasmids to chromosomes, suggesting that IS*26*-*bla*$_{CTX-M}$ brackets could be used to study *bla*$_{CTX-M}$ transmission between humans, domestic animals, and the environment.

IMPORTANCE The horizontal gene transfer events are the major contributors to the current spread of CTX-M-encoding genes, the most common extended-spectrum β-lactamase (ESBL), and many clinically crucial antimicrobial resistance genes (AMR). This study presents evidence of the critical role of IS*26* transposable element for the mobility of *bla*$_{CTX-M}$ gene among *Escherichia coli* isolates from children and domestic animals in the community. We suggest that the nucleotide sequences of IS*26*-*bla*$_{CTX-M}$ could be used to study *bla*$_{CTX-M}$ transmission

between humans, domestic animals, and the environment, since understanding of the dissemination patterns of AMR genes is critical to implement effective measures to slow down the dissemination of these clinically important genes.

KEYWORDS *Escherichia coli*, *bla*$_{\text{CTX-M,}}$ IS*26*, horizontal gene transfer.

The global spread of antimicrobial resistance (AMR) is an urgent threat that affects human health (1). Of particular concern is the dramatic dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, with *Escherichia coli* carrying CTX-M enzyme being the most common (2, 3). Strains carrying ESBLs are resistant to 3rd or later-generation cephalosporins (4), antimicrobials used in hospitals for patients suffering from life-threatening infections (5).

The origin of CTX-M-encoding genes has been traced to chromosomes of several species of *Kluyvera* genus (6–8) from which these *bla*$_{\text{CTX-M}}$ genes have disseminated to other Gammaproteobacteria; it was detected for the first time in *E. coli* in 1991(9). Currently, there are more than 260 *bla*$_{\text{CTX-M}}$ allelic variants identified and clustered into five groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) based on their amino acid sequences (10, 11). In recent years, CTX-M has been the most common ESBL (2, 3) found in a large number of clinically significant bacteria (12–15), and bacteria from human communities (16, 17), and domestic animals (13, 18).

The rapid dissemination of *bla*$_{\text{CTX-M}}$ genes deserves close attention. In a previous study, we found 16 *E. coli* clonal groups (72 *E. coli* isolates involved, of which 95% carried *bla*$_{\text{CTX-M}}$ genes) associated with either humans or domestic animals in semirural communities in Ecuador from which 21 *E. coli* strain-pairs (14%) showing evidence of recent transmission

between domestic animals and humans (18). In this study, we assessed the contribution of horizontal gene transfer of the *bla*$_{CTX-M}$ genes among *E. coli* from humans and domestic animals in these communities.

RESULTS

From the 20 selected *bla*$_{CTX-M}$ allelic variant carrier *E. coli* isolates, we identified 16 plasmids carrying *bla*$_{CTX-M-55}$ (CTX-M-1 group; n = 9), *bla*$_{CTX-M-65}$ (CTX-M-9 group; n = 5), *bla*$_{CTX-M-27}$ (CTX-M-9 group; n = 2) and four chromosomes that carrying *bla*$_{CTX-M-65}$.

Conjugation experiments

Conjugative assays revealed that all 16 *bla*$_{CTX-M}$ allelic variants carried by plasmids were successfully transferable to the recipient *E. coli* TOP10 strain. All transconjugants showed the ESBL phenotype and were resistant to ampicillin (AM; 10mg), cefazolin (CZ; 30mg) and cefotaxime (CTX; 30mg), but susceptible to amoxicillin-clavulanate (AMC; 20 per 10 micrograms), ciprofloxacin (CIP; 5mg), imipenem (IPM; 10mg), tetracycline (TE;30mg) and trimethoprim-sulfamethoxazole (SXT; 1.25 per 23.75 micrograms) (Supplementary table 1). Consistent with the sequencing results, four *bla*$_{CTX-M}$ gene variant carrier *E. coli* isolates could not produce transconjugants since *bla*$_{CTX-M}$ genes were identified on their chromosomes.

Plasmid sequence analysis

The origins, sizes and replicons of plasmids are shown in Table 1. The backbone structure of plasmids (i.e., replication, maintenance and, plasmid transfer genes) and the synteny were conserved in all plasmids carrying the same *bla*$_{CTX-M}$ allelic variant (Fig. 1; 2 and 3). Plasmids

carrying *bla*$_{CTX-M-65}$, *bla*$_{CTX-M-55}$ or *bla*$_{CTX-M-27,}$ however, formed distinct clusters (Fig. S1). A BLASTn analysis of one plasmid representative of each of our *bla*$_{CTX-M-55}$ clusters (4 in total) showed high identity (99.28–99.86%) and high query coverage (68–100%) with three plasmids in two *E. coli* strains (MG197492.1, source: pig, isolation year: 2014; and MG197502.1, source: human, isolation year: 2013) and a *Klebsiella pneumoniae* strain (CP076034.1, source: human, isolation year: 2022), all from China (Fig. S2). The two plasmids carrying *bla*$_{CTX-M-65}$ (one from each cluster) showed high identity (99.81%) and query coverage (90–100%) with two plasmids in an *E. coli* (CP047572.1, source: human, isolation year: 2019) and a *Salmonella enterica* (CP074344.1, source: human, isolation year: 2010) strains isolated in Singapore and Perú, respectively (Fig. S3). In all cases, the plasmids from GenBank carried the same *bla*$_{CTX-M}$ allelic variant as the plasmids from Ecuador.

Table 1. Length, plasmid types and origin of plasmids and chromosomes carrying *bla*$_{CTX-M}$ genes.

Sequence ID	Origin of *E. coli* isolate	Allelic variant *bla*$_{CTX-M}$	Size (bp)	Plasmid type
p201809183.4	Child	*bla*$_{CTX-M-55}$	70218	IncFII(pHN7A8)
p2018091176.5	Chicken	*bla*$_{CTX-M-55}$	71234	IncFII(pHN7A8) - IncFII(p96A)
p201809183.3	Child	*bla*$_{CTX-M-55}$	71428	IncFII(pHN7A8) - IncFII(p96A)
p2018081440.2	Chicken	*bla*$_{CTX-M-55}$	94949	IncFII(pHN7A8) - IncFII(p96A) - ColE10 - [a]IncN
p2018081445.5	Dog	*bla*$_{CTX-M-55}$	96300	IncFII(pHN7A8) - [a]IncN
p2018082847.3	Dog	*bla*$_{CTX-M-55}$	97825	IncFII(pHN7A8) - IncFII(p96A) - ColE10 - [a]IncN
p201809181.3	Child	*bla*$_{CTX-M-55}$	99774	IncFII(pHN7A8) - IncFII(p96A) - ColE10 - [a]IncN
p2018081457.3	Chicken	*bla*$_{CTX-M-55}$	100093	IncFII(p96A) - IncFIC(FII) - ColE10 - IncN
p2018092531.2	Dog	*bla*$_{CTX-M-55}$	114357	IncFII(pHN7A8) - IncFII(p96A) - IncX1 - IncX9 - ColE10 - IncN
p2018091166.4	Chicken	*bla*$_{CTX-M-65}$	104865	IncIg - IncFII(pECLA)
p2018081441.5	Dog	*bla*$_{CTX-M-65}$	105924	IncI1 - IncFII(pECLA)
p2018092511.2	Child	*bla*$_{CTX-M-65}$	117806	IncIg - IncFII(pECLA)
p2018091864.1	Dog	*bla*$_{CTX-M-65}$	126144	IncIg - IncFII(pECLA)
p2018091135.3	Dog	*bla*$_{CTX-M-65}$	127219	IncIg - IncFII(pECLA)
2018081445.4	Dog	*bla*$_{CTX-M-65}$	4468621	-
2018102322.3	Chicken	*bla*$_{CTX-M-65}$	5174885	-
201810092.3	Child	*bla*$_{CTX-M-65}$	5195143	-
2018081453.2	Chicken	*bla*$_{CTX-M-65}$	5211469	-
p2018090418.2	Child	*bla*$_{CTX-M-27}$	121366	IncFIB(AP001918) – IncFII

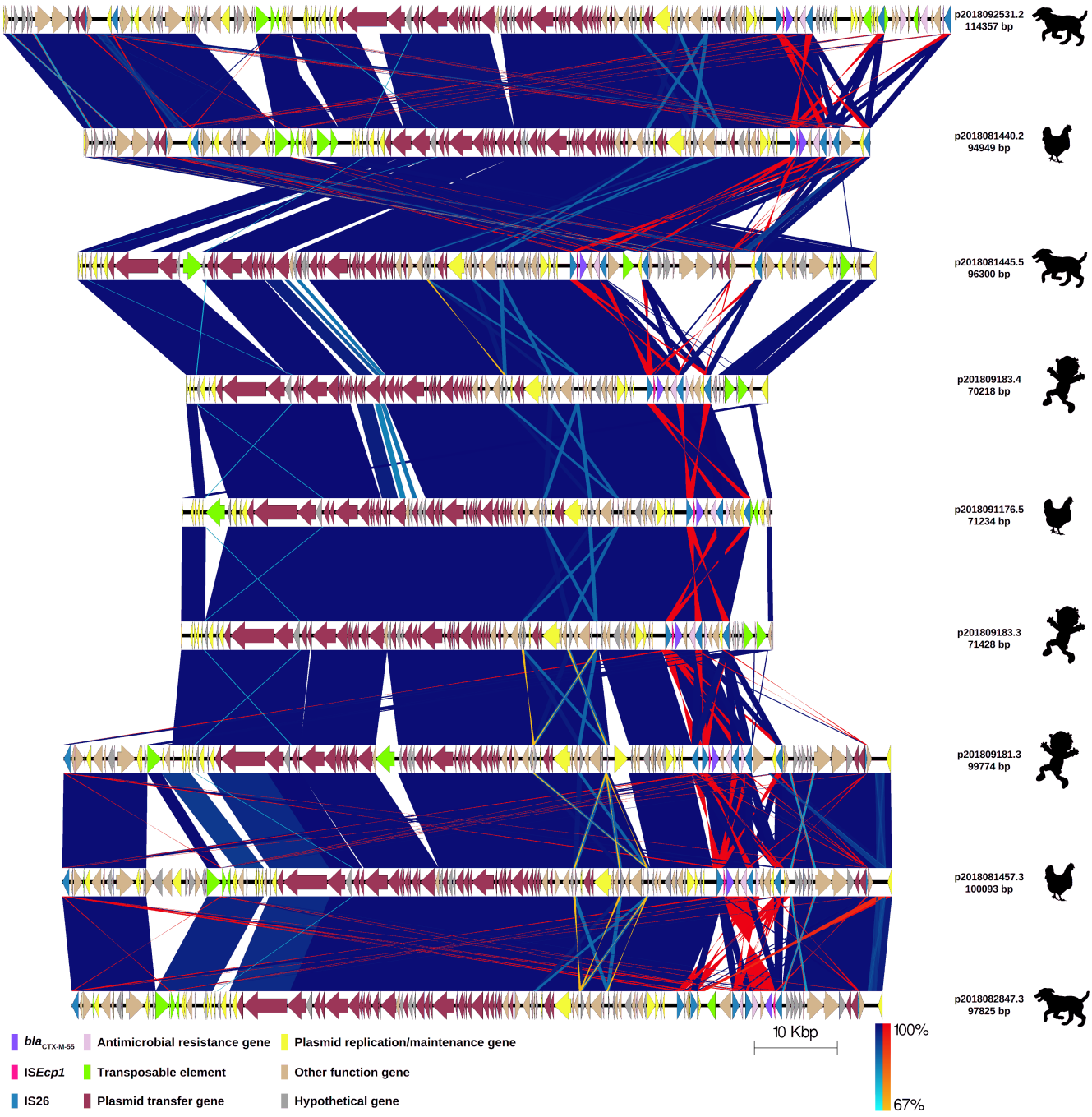

FIG 1. Comparison of nine plasmids carrying *bla*$_{\text{CTX-M-55}}$ gene variant of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from children, chickens, and dogs. Labels show the plasmid ID assigned based on the host ID followed by its isolate number and length of the plasmid carrying the *bla*$_{\text{CTX-M-55}}$ allelic variant. The origin of the isolate harboring the plasmid is shown by a figure in black (child, chicken, and dog). Each plasmid is represented by linear visualization, and coding sequences (CDSs) are represented by arrows. The direction of the arrow indicates the transcription direction of each CDS. CDSs are colored based on their functions. Blue and red shading areas between plasmids indicate the similarity of regions in the same and inverted directions, respectively, according to BLASTn. The percentage of sequence similarity is shown according to a color gradient.

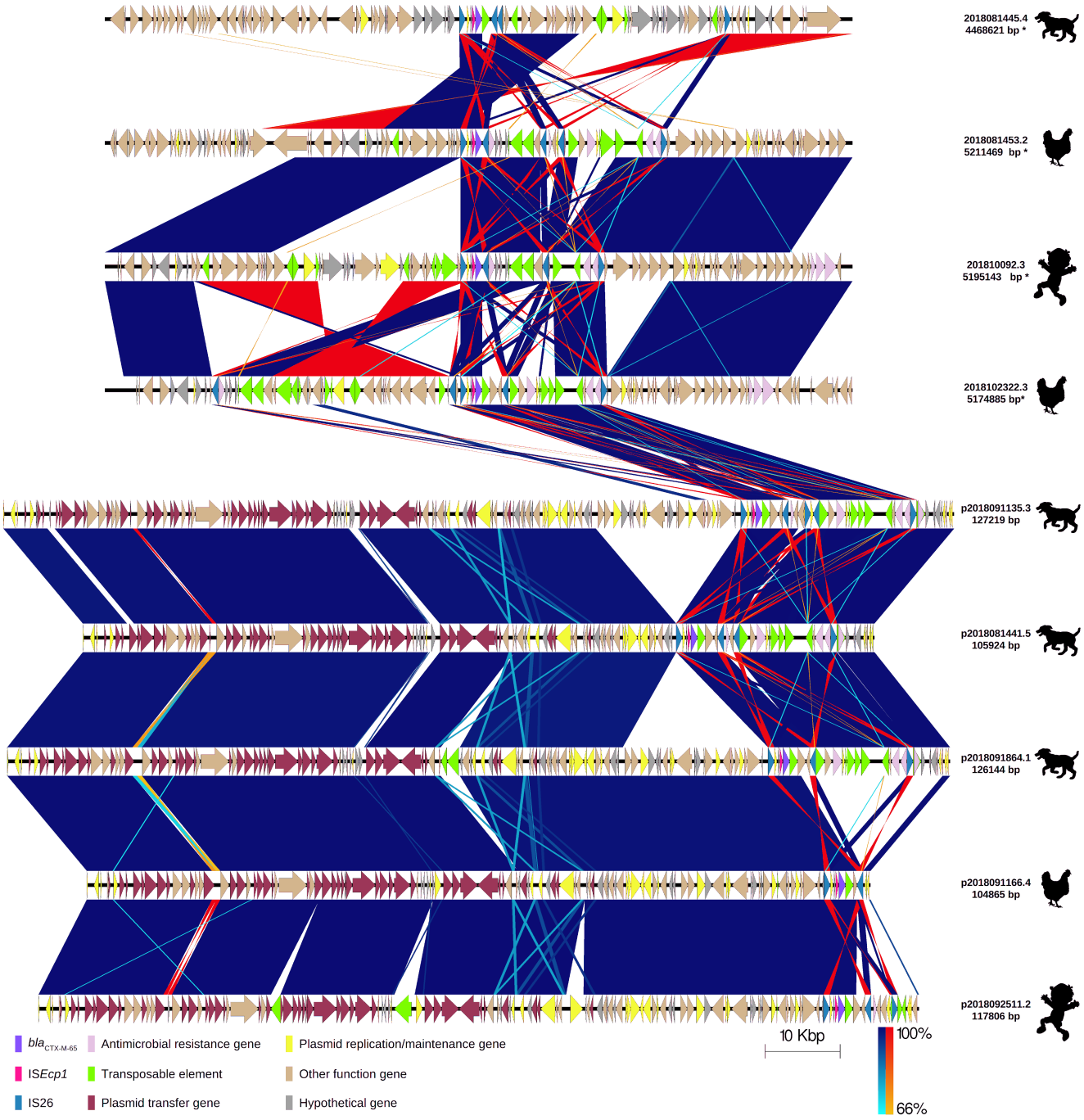

FIG 2. Comparison of four chromosome fragments (100,000 pb) and five plasmids carrying *bla*$_{\text{CTX-M-65}}$ gene variant of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from children, chickens, and dogs. Labels show the plasmid and chromosome ID assigned based on the host ID followed by its isolate number and length of chromosome* and plasmid carrying *bla*$_{\text{CTX-M-65}}$ allelic variant. The origin of the isolate harboring the plasmid is shown by a figure in black (child, chicken, and dog). Each chromosome fragment or plasmid is represented by linear visualization, and coding sequences (CDSs) are represented by arrows. The direction of the arrow indicates the transcription direction of each CDS. CDSs are colored based on their functions. Blue and red shading areas between sequences indicate the similarity of regions in the same and inverted directions, respectively, according to BLASTn. The percentage of sequence similarity is indicated according to a color gradient.

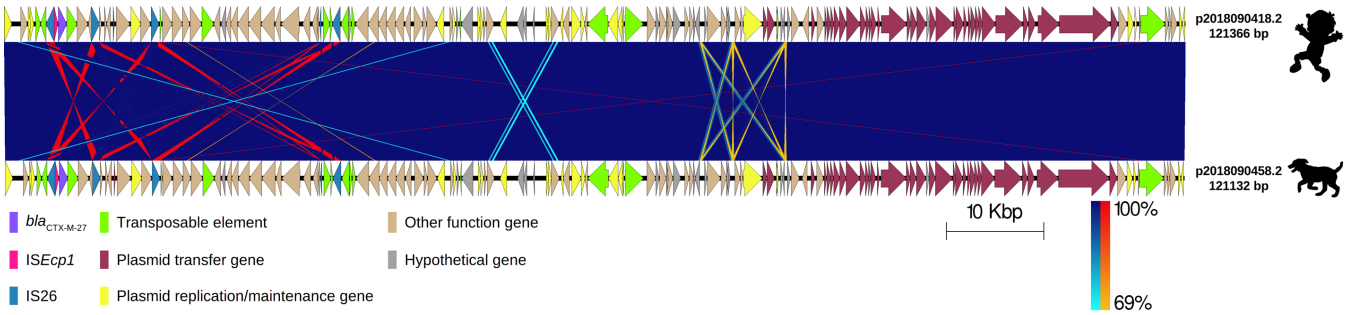

FIG 3. Comparison of two plasmids carrying *bla*$_{\text{CTX-M-27}}$ gene variant of extended-spectrum β-lactamase-producing *Escherichia coli* isolates that were part of a clonal relationship with 0 SNPs in their core genomes. Labels show the plasmid ID assigned based on the host ID followed by its isolate number and length of the plasmid carrying the *bla*$_{\text{CTX-M-27}}$ allelic variant. The origin of the isolate harboring the plasmid is shown by a figure in black (child and dog). Each plasmid is represented by linear visualization and coding sequences (CDSs) represented by arrows. The direction of the arrow indicates the transcription direction of each CDS. CDSs are colored based on their functions. Blue and red shading areas between plasmids indicate the similarity of regions in the same and inverted directions, respectively, according to BLASTn. The percentage of sequence similarity is indicated according to a color gradient.

Genetic environment of *bla*$_{\text{CTX-M}}$ genes

In all plasmids, the *bla*$_{\text{CTX-M-55}}$ gene was bracketed by two IS*26* transposable elements and located 127 bp downstream of a fragment of IS*ECp1* insertion sequence (243 bp – 14.7% coverage) truncated by IS*26,* and 46 bp upstream of the *wbuC* gene which codes a cupin fold metalloprotein. Downstream of the *wbuC* gene, the Tn*A* and *bla*$_{\text{TEM}}$ gene were found, and both were truncated by IS*26* (Fig. 4). This structure was the same (99% identity) for all the nine *bla*$_{\text{CTX-M-55}}$-carrying plasmids (Fig. 4). We use the term IS*26*-*bla*$_{\text{CTX-M}}$ bracket to indicate the nucleotide sequence containing the *bla*$_{\text{CTX-M}}$ gene that is flanked by two IS*26*s. In the plasmid from one isolate (ID: 2018082847.3), the IS*26*-*bla*$_{\text{CTX-M}}$ bracket, containing *bla*$_{\text{CTX-M-55}}$ and identical genes, was in opposite direction indicating inversion caused most probably by recombination or transposition of both IS*26*s (Fig. 1). The nine *bla*$_{\text{CTX-M-65}}$ gene variants were detected in five plasmids and four chromosomes. Similar to the case of *bla*$_{\text{CTX-M-55}}$, the *bla*$_{\text{CTX-}}$

$_{M\text{-}65}$ gene in all cases was bracketed by two IS*26*s (Fig. 5): In three plasmids (from 2018091135.3, 2018091864.1, and 2018081441.5 isolates) and one chromosome (from 2018102322.3 isolate), IS*26*-*bla*$_{CTX\text{-}M}$ brackets contained the same genes: *fipA* gene encoding a conjugal transfer inhibition protein, a hypothetical gene, IS*Ecp1* fragment, *bla*$_{CTX\text{-}M\text{-}65}$ gene, IS*102* insertion sequence, a gene encoding a TonB-dependent receptor, and a gene encoding PAS domain-containing protein; in the two chromosomes (from 2018081453.2 and 201810092.3 isolates) the IS*26*-*bla*$_{CTX\text{-}M}$ bracket contained fewer of the same genes in the same order: *fipA* gene encoding a conjugal transfer inhibition protein, the hypothetical gene, IS*Ecp1* fragment, *bla*$_{CTX\text{-}M\text{-}65}$ gene. Although the IS*26*-*bla*$_{CTX\text{-}M}$ bracket contained the same genes and in the same order, some of the genes were located at different distances from each other: IS*26* – *fipA* gene (44 bp: 2018081453.2, 201810092.3, p2018102322.3, p2018091864.1, and p2018081441.5; 43 bp: p2018091135.3), *fipA* gene – hypothetical gene (64 bp: 2018081453.2, 201810092.3, p2018091135.3, and p2018091864.1; 63 bp: p2018102322.3 and p2018081441.5), gene encoding a TonB-dependent receptor – gene encoding PAS domain-containing protein (67 bp: p2018091135.3 and p2018081441.5; 68 bp: p2018091864.1; 24 bp: p2018102322.3), gene encoding PAS domain-containing protein – IS*26* (14 bp: p2018091135.3, p2018091864.1 and p2018081441.5; 57bp: p2018102322.3).

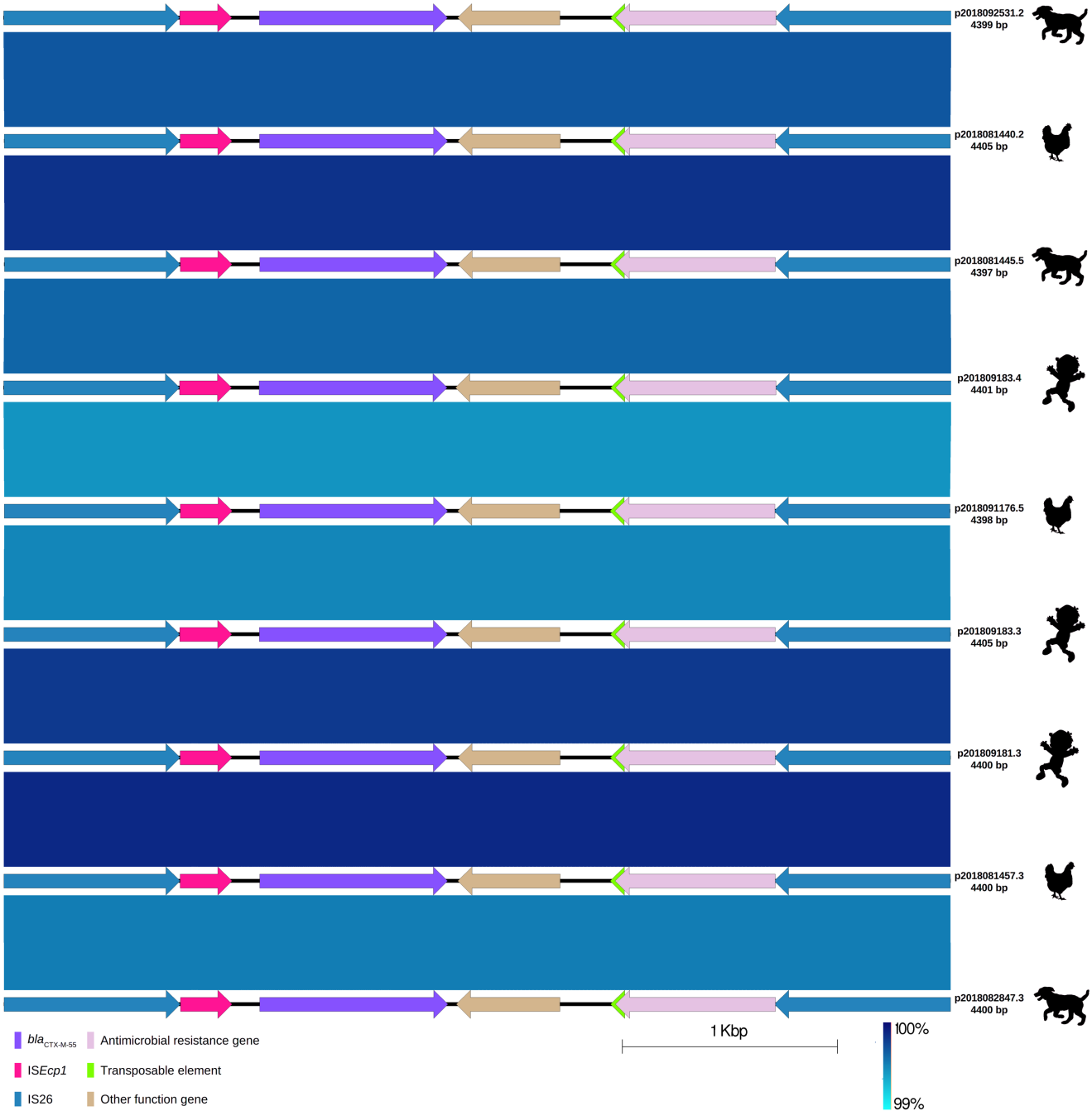

FIG 4. Comparison of nine IS*26*-*bla*$_{\text{CTX-M-55}}$ brackets of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from children, chickens, and dogs. Labels show plasmid ID (harboring the IS*26*-*bla*$_{\text{CTX-M}}$ bracket) assigned based on the host ID followed by its isolate number and length of the IS*26*-*bla*$_{\text{CTX-M-55}}$ bracket. The origin of the isolate harboring the plasmid is shown by a figure in black (child, chicken, and dog). Each IS*26*-*bla*$_{\text{CTX-M-55}}$ bracket is represented by linear visualization, and coding sequences (CDSs) are represented by arrows. The direction of the arrow indicates the transcription direction of each CDS. CDSs are colored based on their functions. Blue shading areas between plasmids indicate the similarity of regions in the same direction according to BLASTn. The percentage of sequence similarity is indicated according to a color gradient.

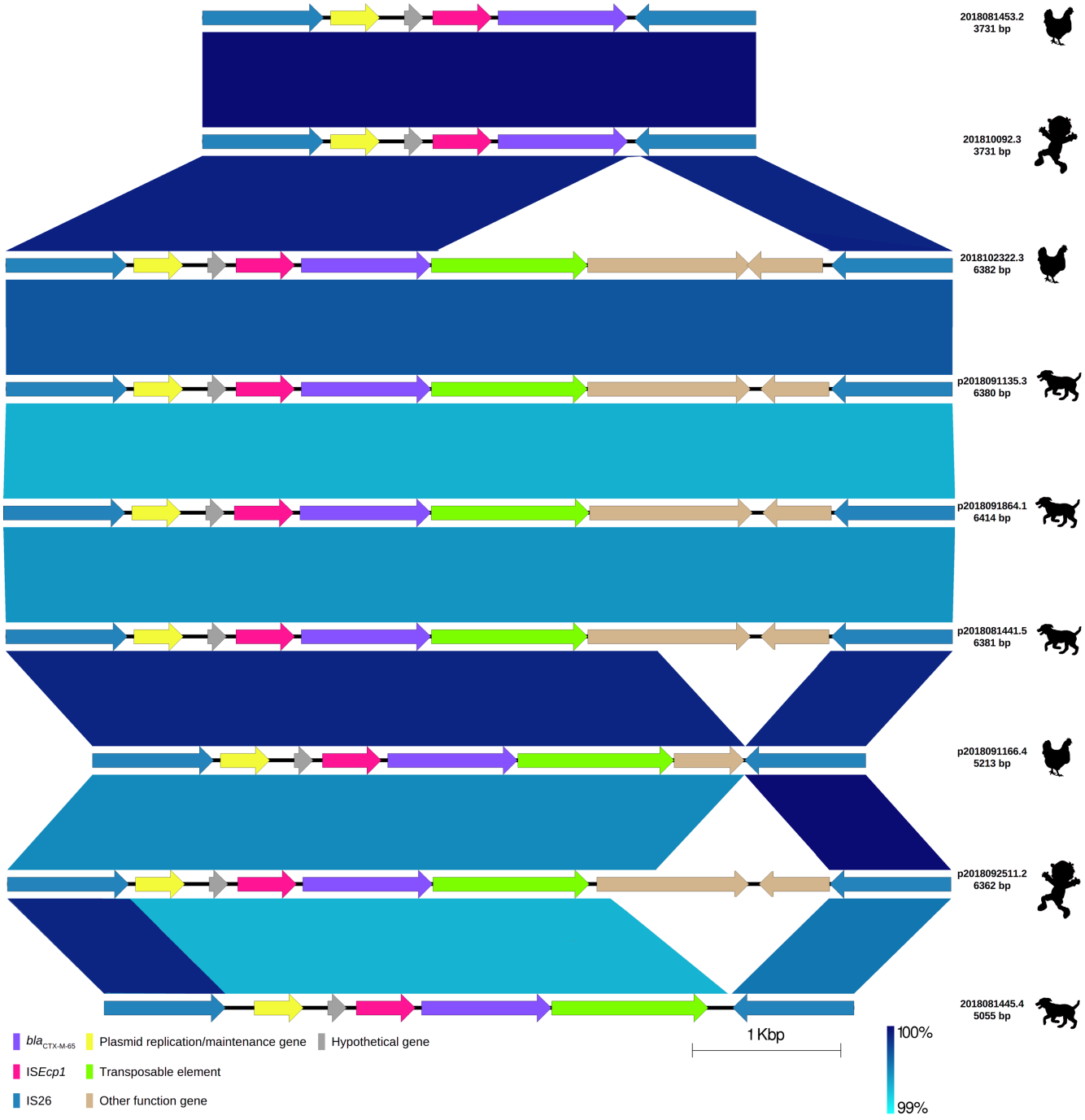

FIG 5. Comparison of nine IS*26*-*bla*$_{\text{CTX-M-65}}$ brackets of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from children, chickens, and dogs. Labels show the plasmid or chromosome ID (harboring the IS*26*-*bla*$_{\text{CTX-M}}$ bracket) assigned based on the host ID followed by its isolate number and length of the IS*26*-*bla*$_{\text{CTX-M-65}}$ bracket. The origin of the isolate harboring the plasmid or chromosome is shown by a figure in black (child, chicken, and dog). Each IS*26*-*bla*$_{\text{CTX-M-65}}$ bracket is represented by linear visualization, and coding sequences (CDSs) are represented by arrows. The direction of the arrow indicates the transcription direction of each CDS. CDSs are colored based on their functions. Blue shading areas between plasmids indicate the similarity of regions in the same direction according to BLASTn. The percentage of sequence similarity is indicated according to a color gradient.

IS*26*-*bla*$_{CTX-M}$ bracket similarity search

The IS*26*-*bla*$_{CTX-M-55}$ bracket from p2018081440.2 showed similarity with sequences from six different species in the GenBank: *E. coli* (*n* = 69), *Klebsiella pneumoniae* (*n* = 12), *Salmonella enterica* (*n* = 10), *Salmonella* sp. (*n* = 5), *Escherichia albertii* (*n* = 2), *Acinetobacter baumannii* (*n* = 1), and *Citrobacter freundii* (*n* = 1), with a query coverage of 100% and identities ranging from 99.84% to 99.91%. The IS*26*-*bla*$_{CTX-M-65}$ bracket from p2018091135.3 showed similarity with sequences from four different species: *S. enterica* (*n* = 46), *Proteus mirabilis* (*n* = 26), *E. coli* (*n* = 24), and *K. pneumoniae* (*n* = 3), with a query coverage between 95% and 100% and identities between 96.33% and 99.67%. Whereas the IS*26*-*bla*$_{CTX-M-65}$ bracket from p201810092.3 showed similarities with sequences from eight different species: *E. coli* (*n* = 46), *P. mirabilis* (*n* = 24), *S. enterica* (*n* = 21), *K. pneumoniae* (*n* = 3), *Kluyvera intermedia* (*n* = 2), *Enterobacter hormaechei* (*n* = 1), and *Escherichia fergusonii* (*n* = 1), and *Klebsiella aerogenes* (*n* = 1), with a query coverages ranging between 56% and 100% and identities between 99.76% and 99.95%.

To assess the SNPs in IS*26*-*bla*$_{CTX-M}$ brackets, we carried out pairwise SNPs analysis between the three brackets with the sequences showing highest similarity in BLASTn analyses. For IS*26*-*bla*$_{CTX-M-55}$ bracket from p2018081440.2 we identified 88 (88%) that presented 2 SNPs and 12 (12%) showing 3 SNPs. For IS*26*-*bla*$_{CTX-M-65}$ bracket from p2018091135.3, the pairwise SNPs analysis showed 34 (34.34%), 56 (56.57%), 7 (7.07%) and 2 (2.02%) sequences with 1, 2, 3 and 4 SNPs, respectively. Interestingly, 1 (1.01%) and 98 (98.99%) from the 99 match sequences to IS*26*-*bla*$_{CTX-M-65}$ bracket from p201810092.3 showed 1 and 0 SNPs, respectively.

Phylogenetic analysis of Plasmids and IS*26*-*bla*$_{CTX-M}$ brackets

To explore the possibility of IS*26*-*bla*$_{CTX-M}$ bracket mobilization among different plasmids, we compared the topology of maximum likelihood phylogenetic trees of the plasmids and the IS*26*-*bla*$_{CTX-M}$ brackets carrying either *bla*$_{CTX-M-65}$ or *bla*$_{CTX-M-55}$. Even though some plasmid clustering was concordant with IS*26*-*bla*$_{CTX-M}$ brackets (Examples: plasmids p2018091166.4, p2018081441.5 show a common ancestor and p2018082847.3, p201809181.3 also share a common ancestor), there were many cases where the clustering of plasmids and IS*26*-*bla*$_{CTX-M}$ brackets were discordant (Examples: plasmids p2018092531.2 and p2018081440.2 share a recent common ancestor while their IS*26*-*bla*$_{CTX-M}$ brackets share recent ancestor with IS*26*-*bla*$_{CTX-M}$ brackets from other plasmids p2018092531.2 with p201809181.3, and p2018081440.2 with 2018091176.5) (Fig. 6 and 7). These results suggest that many plasmids have not co-evolved, for some time, with their respective IS*26*-*bla*$_{CTX-M}$ brackets.

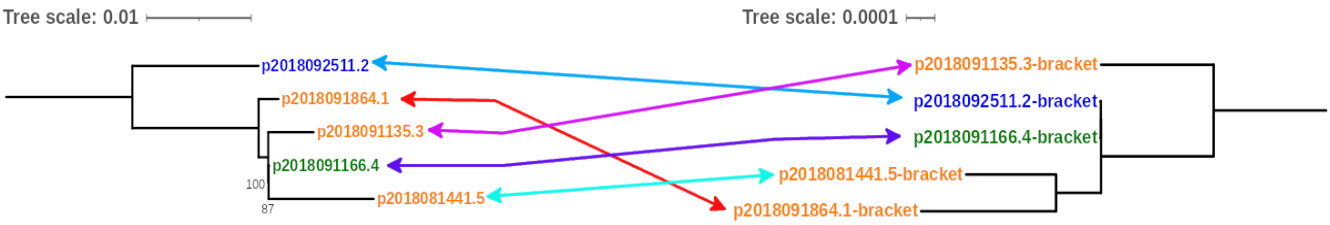

FIG 6. Comparative phylogenetic analysis of complete sequences of plasmids carrying *bla*$_{CTX-M-65}$ allelic variant with their harbored IS*26*-*bla*$_{CTX-M}$ bracket. The evolutionary history was inferred using maximum-likelihood phylogenetics with a general time reversible tree built using the genetic distance. The phylogenetic tree on the left was based on complete sequences of plasmids, whereas the tree on the right was based on IS*26*-*bla*$_{CTX-M}$ sequences. Labels show the isolate ID assigned based on the host ID followed by its isolate number. The origin of the isolate harboring the plasmid is indicated by font colors (child: blue; dog: orange; chicken: green). Colored arrows relate the plasmid to its corresponding IS*26*-*bla*$_{CTX-M}$ bracket. Bootstrap values (>80) based on 100 replications are shown at the tree nodes.

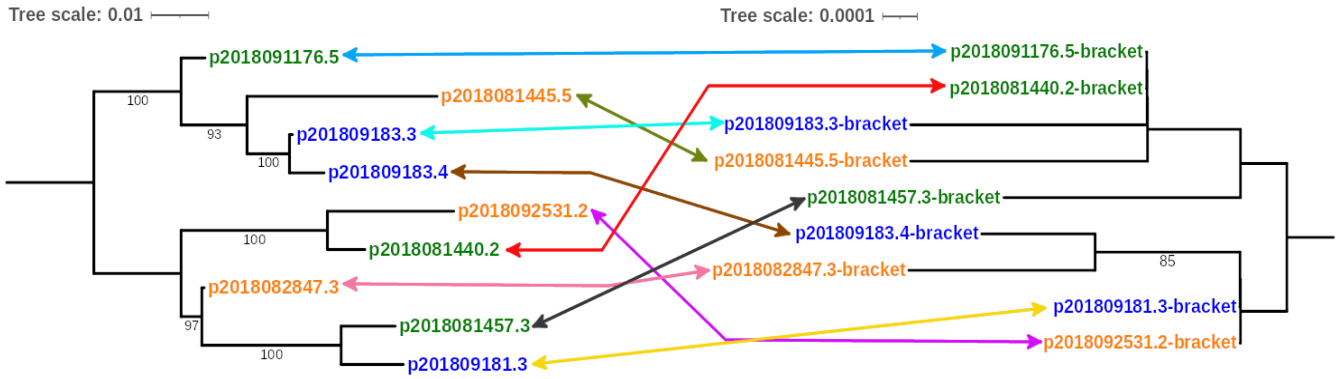

FIG 7. Comparative phylogenetic analysis of complete sequences of plasmids carrying *bla*$_{\text{CTX-M-55}}$ allelic variant with their harbored IS*26*-*bla*$_{\text{CTX-M}}$ bracket. The evolutionary history was inferred using maximum-likelihood phylogenetics with a general time reversible tree built using the genetic distance. The phylogenetic tree on the left was based on complete sequences of plasmids, whereas the tree on the right was based on IS*26*-*bla*$_{\text{CTX-M}}$ sequences. Labels show the isolate ID assigned based on the host ID followed by its isolate number. The origin of the isolate harboring the plasmid is indicated by font colors (child: blue; dog: orange; chicken: green). Colored arrows relate the plasmid to its corresponding IS*26*-*bla*$_{\text{CTX-M}}$ bracket. Bootstrap values (>80) based on 100 replications are shown at the tree nodes.

Plasmid evolutionary rate

To determine the rate of plasmid (carrying *bla*$_{\text{CTX-M}}$ genes) evolution, we took advantage of four clonal *E. coli* strains (2018090418.2 and 2018090458.2: 0 single-nucleotide polymorphisms (SNPs); 2018091135.3 and 2018081441.5: 90 SNPs) isolated during the same period in the same community (18). This plasmid comparison showed a highly conserved structure with an extremely high nucleotide identity (28 SNPs). There were neither plasmid rearrangements, gene insertions, or deletions for plasmids from *E. coli* strains with 0 SNPs in their core genomes. The regions with lower similarity (69%) corresponded to duplicated sequences of hypothetical genes and intergenic spaces. The plasmid sizes were 121366 bp and 121132 bp for plasmids p2018090418.2 and p2018090458.2, respectively (Fig. 3 and Table S2). IS26 also bracketed the two *bla*$_{\text{CTX-M-27}}$ genes, and this region presented 100% identity.

The two plasmids carrying *bla*$_{\text{CTX-M-65}}$ from *E. coli* strains with 90 SNPs in their core genomes also showed a conserved structure with high nucleotide identity (209 SNPs). There were no rearrangements found, however, gene insertion and deletion regions were identified. The lower similarity (71%) corresponded to duplicated sequences of IS*26* and intergenic spaces. The plasmid sizes were 127219 bp and 105924 bp from 2018091135.3 and 2018081441.5 isolates, respectively (Fig. 2 and Table S2).

To further strengthen the results of this analysis, we determined the number of SNPs and length differences between plasmids sequenced in duplicate and the thresholds to define variations due to inherent variations of sequencing and bioinformatics analyses. The mean SNP difference was 0.06%, and mean length difference was 0.10% (Table S2).

MATERIALS AND METHODS

ESBL-producing *E. coli* whole genomes

In a previous study that aimed to study the transmission of cephalosporin-resistant *E. coli* between domestic animals and humans, we analyzed *E. coli* strains that showed high chromosomal similarity (18). To study horizontal gene transfer, we selected 125 ESBL-producing *E. coli* (from domestic animals and humans) carrying the most common *bla*$_{\text{CTX-M}}$ allelic variants identified in these communities (18). We used ResFinder database (19), with 90% minimum match and 60% minimum length) (18). Sixty-nine carried the *bla*$_{\text{CTX-M-55}}$ allelic variant (children = 22, dogs = 20, chickens = 27) and 56 carried the *bla*$_{\text{CTX-M-65}}$ allelic variant (children = 7, dogs = 34, chickens = 15).

Characterization of *bla*$_{CTX-M}$ carrier contigs

Mobile genetic elements of 125 *bla*$_{CTX-M}$ gene variant carrier contigs were identified using the command line version of MobileElementFinder 1.0.3 (20) with 80% minimum match and 10% minimum length. Then, *bla*$_{CTX-M-55}$ and *bla*$_{CTX-M-65}$ allelic variant carrier contigs were separately aligned in Unipro UGENE (21) to establish it into groups based on the similarity of their nucleotide sequences. The *bla*$_{CTX-M-55}$ gene carrier contigs were classified into six groups, while the *bla*$_{CTX-M-65}$ gene carrier contigs were classified into eight groups. From the established groups in which there were contigs from ESBL-producing *E. coli* whole genomes isolated from more than one species, we randomly selected one isolate from each species for further analyses. Additionally, to determine the rate of plasmid (carrying *bla*$_{CTX-M}$ genes) evolution, we choose four contigs of four different ESBL-producing *E. coli* whole genomes, two with 0 SNPs in their core genomes (carrying *bla*$_{CTX-M-27}$ allelic variant) and two with 90 SNPs in their core genomes (carrying *bla*$_{CTX-M-65}$ allelic variant) (18).

DNA extraction of *bla*$_{CTX-M}$ gene carrier plasmids

Each of the 20 selected *bla*$_{CTX-M}$ allelic variant carrier *E. coli* isolates was reactivated on MacConkey Lactose agar (Difco) supplemented with ceftriaxone (2 mg/L) overnight at 37 °C, after which one colony was selected and inoculated into 2 mL of Lysogeny Broth (LB) supplemented with ceftriaxone (2 mg/L) with shaking at 250 rpm at 37 °C for 9 h. Then, 3 mL of fresh LB media with antibiotic were added to the culture at 37 °C for 12 to 16 h while shaking at 250 rpm. Plasmid extraction from the 20 isolates was performed in duplicate using Pure Yield^{TM} Plasmid Miniprep System (Promega) according to the protocol provided by the manufacturer. Duplicates were placed into a single microtube before being freeze-dried and

resuspended in nuclease-free water to achieve a minimum plasmid DNA concentration of 53 ng/µL. Extracted plasmid DNA concentrations were measured using a Qubit™ 1X dsDNA High Sensitivity assay kit and a Qubit 4.0 fluorometer (Thermo Fisher Scientific).

Genomic DNA extraction

For the four isolates whose sequences could not be circularized or in which the *bla*$_{\text{CTX-M}}$ allelic variant could not be identified after sequencing and assembly, genomic DNA extraction was performed using 12 to 16 h cultures obtained as mentioned above, using DNeasy Blood & Tissue kit (Qiagen). DNA was eluted in nuclease-free water, and a minimum DNA concentration of 53 ng/µL was obtained, measured using a Qubit™ 1X dsDNA High Sensitivity assay kit and a Qubit 4.0 fluorometer (Thermo Fisher Scientific).

Conjugation experiments

Conjugation assays were performed to evaluate the conjugative capacity of *bla*$_{\text{CTX-M}}$ carrier plasmids. The 20 selected *bla*$_{\text{CTX-M}}$ allelic variant carrier *E. coli* isolates were used as donors, and *E. coli* TOP10 (Invitrogen) resistant to rifampin as the recipient (22). Prior to conjugation experiments the phenotypic AMR profile of each donor strains was confirmed against the same 12 antimicrobials used in our previous study (18) by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (23). Among the 12 antimicrobials we used ceftazidime (CAZ; 30µg), cefotaxime (CTX; 30µg), cefepime (FEP; 30µg) and amoxicillin-clavulanate (AMC; 20 per 10µg), with which we carried out the double-disk synergy test (24). Phenotypic expression of ESBL was evaluated by placing a disk of AMC surrounded by disks of CAZ, CTX and FEP (30 mm apart, center to center). An extension of the

edge of the CAZ, CTX or FEP inhibition zone toward AMC disk as a keyhole effect was interpreted as positive for the ESBL phenotype (24, 25). For each conjugation experiment, the donor and recipient strains were grown in LB at 37°C for 18 h, and the strains in the logarithmic growth phase were mixed and incubated at 37°C for 18 h. Transconjugants were selected by the spread plate method onto LB agar containing ceftriaxone (2 mg/L) and rifampin (100 µg/ml) as previously described (22). The phenotypic expression of ESBL by DDST and antimicrobial phenotypic profile by disk diffusion of transconjugants was evaluated to determine the acquired antimicrobial resistance.

MinION library preparation and sequencing

According to the manufacturer's instructions, library preparation was performed using the Rapid Barcoding Sequencing Kit (SQK-RBK004) (Oxford Nanopore Technologies). The constructed libraries were loaded into R9.4.1 (FLO-MIN106D) flow cells and sequenced on a MinION Mk1B sequencing device for approximately 24 hours using the MinKNOW software 22.03.5 (Oxford Nanopore Technologies). We sequenced a random selection of three plasmid DNA samples twice, obtained from the same bacterial cultures, to determine the intrinsic variations of sequencing and bioinformatic analyses. Basecalling was carried out with Guppy 6.0.6 (https://community.nanoporetech.com) in a fast basecalling model. Raw data were demultiplexed, and adapters and barcodes were trimmed using Porechop 0.2.4 with default parameters (26). Then, raw reads were filtered with Filtlong 0.2.1 using a minimum read length threshold of 1 kbp and keeping 95% of the best reads (measured by bases). Filtered reads metrics were assessed with NanoPlot 1.40.0 (27).

Assembly of plasmids and chromosomes carrying *bla*$_{CTX-M}$ gene

De *novo* assembly of complete plasmids and chromosomes with filtered reads was carried out using Flye assembler 2.8.1-b1676 (28). Different assembly parameters were evaluated to optimize the assembly of the circular sequences of interest due to the unknown plasmid size and possible contamination with chromosomal DNA in the plasmid DNA samples. The genome-size option was set at 0.1, 1, 2, 3, 4, and 5 m each, with the asm-coverage option set at 10, 15, 20, 30, 40, and 50, with all combinations. The plasmids option was specified to allow recovery of unassembled short plasmids. Additionally, the meta-assembly option (29) was also assessed.

***bla*$_{CTX-M}$ gene variant carrier plasmids and chromosomes annotation**

AMR genes and plasmid types were identified with Resfinder (19) and PlasmidFinder (30) databases, respectively, using ABRicate tool 1.0.1 (31) in all plasmids and chromosomes circularized. Each *bla*$_{CTX-M}$ gene variant carrier plasmid and chromosome was rotated with the task fixstart of Circlator tool 1.5.5 (32) and a fasta file with 7171 ancestral sequences of most common replication initiators (33) to fix the start position of each plasmid and chromosome. Since plasmids usually have more than one replication initiator, they were aligned with MAFFT algorithm and manually modified to establish the same replication origin in cases where possible in Unipro UGENE 40.1 (21). For the plasmid sequences of the DNA samples sequenced twice, we used Unipro UGENE to align them and obtain the consensus sequences using the Levitsky algorithm. The number of SNPs between plasmids sequenced twice and between plasmids from clonal *E. coli* strains was determined using Snippy 4.6.0 (34). Each *bla*$_{CTX-M}$ gene variant carrier plasmid and chromosome was annotated with the National Center for

Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (35). The output GenBank file was manually curated using data obtained from different annotation tools. Mobile genetic elements were identified using the command line version of MobileElementFinder 1.0.3 (20), and AMR genes and plasmid types were again predicted after rotation of sequences with Resfinder and PlasmidFinder databases, respectively. The genomic structure comparison among plasmids and chromosomes and among IS*26*-*bla*$_{\text{CTX-M}}$ brackets was performed according to BLASTn using Easyfig 2.2.2 (36).

Similarity of IS*26*-*bla*$_{\text{CTX-M}}$ brackets

The IS*26*-*bla*$_{\text{CTX-M}}$ bracket sequences similarity search was carried out with IS*26*-*bla*$_{\text{CTX-M-55}}$ bracket (from p2018081440.2), and with the two most common IS*26*-*bla*$_{\text{CTX-M-65}}$ brackets identified (from p2018091135.3 and p201810092.3 respectively), using BLASTn without inclusion or exclusion parameters. All 100 match sequences for each of the three IS*26*-*bla*$_{\text{CTX-M}}$ brackets, excluding synthetic constructs, were selected. We used Unipro UGENE 40.1 to convert sequences to their reverse complement as necessary to ensure that all selected sequences are in the same orientation respect to the IS*26*-*bla*$_{\text{CTX-M}}$ brackets. Then, the number of SNPs between each of the selected sequences and their respective IS*26*-*bla*$_{\text{CTX-M}}$ bracket was determined using Snippy 4.6.0.

Phylogenetic analyses

To investigate the possibility of IS*26*-*bla*$_{\text{CTX-M}}$ bracket mobilization among different plasmids, we constructed maximum likelihood phylogenetic trees of the plasmids and the IS*26*-*bla*$_{\text{CTX-M}}$ brackets carrying *bla*$_{\text{CTX-M-65}}$ or *bla*$_{\text{CTX-M-55.}}$ Due to inverted sequences in plasmids that

concealed their phylogenetic relationships, we identified inverted DNA sequences using Easyfig 2.2.2 (36), and we manually placed these sequences in the same direction using Unipro UGENE 40.1 (21) before phylogenetic tree construction. We also carried out BLASTn analyses of one representative plasmid of each phylogenetic tree cluster obtained to identify the best match plasmids. Additionally, BLASTn analyses of IS*26*-*bla*$_{\text{CTX-M-55}}$ bracket, and the most common IS*26*-*bla*$_{\text{CTX-M-65}}$ bracket identified were performed to establish the best match plasmid harbored by *Kluyvera* spp. Then, from the *Kluyvera* plasmid sequence more similar to the IS*26*-*bla*$_{\text{CTX-M-65}}$ bracket, we carried out a new BLASTn comparison to select the four best match plasmid sequences to include them in a phylogenetic tree based on all of our plasmids carrying either *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-27}}$. All maximum likelihood phylogenetic trees were performed with the general time reversible (GTR) model using RaxML-NG 0.6.0 (37). The visualization and edition of phylogenetic trees were carried out using iTOL v6 (38) and GIMP 2.10 (https://www.gimp.org), respectively.

Data Availability

Assembled plasmids and chromosomes were deposited into the NCBI database under accession number: PRJNA973083.

DISCUSSION

Our results suggest that IS*26* mobilizes *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{CTX-M-55}}$, and *bla*$_{\text{CTX-M-27}}$ allelic variants among different plasmids (Fig. 1; 2 and 3). Even though some plasmids carrying the same *bla*$_{\text{CTX-M}}$ gene shared a more recent ancestor, which may suggest plasmid co-evolution with

bla$_{CTX-M}$ genes (Fig. S1), the phylogeny of the IS*26*-*bla*$_{CTX-M}$ did not correspond to plasmid phylogeny (Fig. 6 and 7). Additional evidence of IS*26* contribution in *bla*$_{CTX-M}$ mobility is the presence of the identical IS*26*-*bla*$_{CTX-M-65}$ bracket in the plasmids of three different isolates and the chromosome of another isolate (Fig. 5). We also found evidence of different evolutionary trajectories of *bla*$_{CTX-M}$ genes and plasmids; genes *bla*$_{CTX-M-27}$ and *bla*$_{CTX-M-65}$ belong to the phylogenetic group 1 whereas *bla*$_{CTX-M-55}$ belong to phylogenetic group 9 (8). However, our results show that plasmids carrying *bla*$_{CTX-M-27}$ share a more recent common ancestor with plasmids carrying *bla*$_{CTX-M-55}$ than with plasmids carrying *bla*$_{CTX-M-65}$ (Fig. S1). These observations suggest that the plasmids have exchanged IS*26*-*bla*$_{CTX-M}$ brackets (through transposition or recombination) throughout their evolution. The large divergence in plasmids carrying *bla*$_{CTX-M}$ genes is consistent with the notion that *bla*$_{CTX-M}$ genes were associated with different plasmids which existed before the use of 3rd generation cephalosporins (14) (Table 1).

Our findings are also consistent with recent reports showing that IS*26* is extremely active, as transposable elements, mobilizing many important AMR genes (39). In our study, however, the IS*26*-*bla*$_{CTX-M-55}$ brackets carried a fragment of the *bla*$_{TEM}$ gene (in addition to the *bla*$_{CTX-M}$ gene); seven of the nine plasmids carrying *bla*$_{CTX-M-55}$ allelic variant showed the *fosA3* gene (coding for fosfomycin resistance) in the vicinity, while another plasmids showed a fragment of the *mef(B)* (coding for a macrolide efflux pump) in the vicinity (Figure 1). Similarly, IS*26*-*bla*$_{CTX-M-65}$ brackets showed more AMR genes: *fosA3*, *floR* (coding for chloramphenicol), *aph(4)-Ia* (coding for an aminoglycoside phosphotransferase), *aac(3)-Iva* (coding for gentamicin), *ant(3")-Ia* (coding for streptomycin) in the vicinity (Fig. 2).

Even though we were not able to observe direct transmission of a plasmid between *E. coli* from domestic animals and humans, all the plasmids carrying *bla*$_{CTX-M}$ genes were conjugable, and domestic animals and humans shared many (65%, 13 of 20) of the IS*26*-*bla*$_{CTX-M}$ brackets. These findings suggest that horizontal gene transfer events of diverse plasmids and *bla*$_{CTX-M}$ genes outnumber clonal transmission events (among domestic animals and humans), as we found 14% of *bla*$_{CTX-M}$ *E. coli* strains presented evidence of recent transmission between humans and domestic animals (18). These results suggest that the IS*26*-*bla*$_{CTX-M}$ bracket involves a complex multi-step process of horizontal gene transfer in which transposons mobilize the *bla*$_{CTX-M}$ among plasmids or from plasmids to chromosomes. These results agree with previous observations that some *bla*$_{CTX-M}$ gene variants (and their contiguous regions) were associated with specific environments in Ecuador (40). In these cases, the only evidence of AMR-gene transmission is the presence of the highly similar IS*26*-*bla*$_{CTX-M}$ brackets in different isolates in an epidemiological context compatible with this transmission. We acknowledge that due to the fact that the *E. coli* isolates were obtained from the same geographic region in Ecuador (18), the results may not be generalizable to other countries, although our IS*26*-*bla*$_{CTX-M}$ brackets were highly similar to sequences found in plasmids and chromosomes from GenBank, suggesting that IS*26*-*bla*$_{CTX-M}$ brackets may play an important role in the dissemination of *bla*$_{CTX-M}$ gene through several bacterial species in different geographic regions.

In conclusion, the prevalence of CTX-M enzymes has increased dramatically since the mid to late 2000s (3). ESBL-encoding genes have been identified in plasmids, present in *E. coli* strains isolated before using 3rd generation cephalosporins in 1981, suggesting that the *E. coli* acquisition of these genes had occurred in multiple independent events (14). The IS*26*

transposable element is critical for the current mobility of these and other clinically crucial AMR genes. Our study suggests that the nucleotide sequences of IS*26*-*bla*$_{CTX-M}$ brackets could be an important genetic structure to study *bla*$_{CTX-M}$ transmission between humans, domestic animals, and the environment. We provide evidence for the complexity of the *bla*$_{CTX-M}$ horizontal gene transfer and how this understanding can be applied to determine the dissemination of these genes in any community, animals, or environment. The amplification and sequencing of the DNA inside the brackets may be used to monitor the *bla*$_{CTX-M}$ dynamics (increasing rates, allelic variant replacement, dissemination, etc.). The understanding of the dissemination patterns of AMR genes is critical to implement effective measures to slow down the dissemination of these clinically important genes.

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SUPPLEMENTAL MATERIAL

Table S1. Antimicrobial phenotypic profile of original isolates and their transconjugants.

Isolate ID	CZ		CIP		AM		C		IPM		SXT		GM		CAZ		FEP		CTX		TE		AMC		ESBL
201809183.4	0	R	35	S	0	R	23	S	28	S	27	S	20	S	18	I	18	R	0	R	0	R	19	S	Positive
201809183.4-t	0	R	44	S	0	R	30	S	32	S	34	S	28	S	19	I	22	I	12	R	30	S	20	S	Positive
201809181.3	0	R	14	R	0	R	0	R	29	S	0	R	23	S	24	S	21	I	10	R	0	R	24	S	Positive
201809181.3-t	0	R	44	S	0	R	30	S	33	S	32	S	24	S	21	S	23	I	13	R	30	S	23	S	Positive
2018081457.3	0	R	27	S	0	R	0	R	27	S	17	S	20	S	21	S	20	I	10	R	8	R	23	S	Positive
2018081457.3-t	0	R	44	S	0	R	30	S	33	S	32	S	25	S	22	S	21	I	10	R	30	S	24	S	Positive
2018082847.3	0	R	26	S	0	R	0	R	33	S	0	R	20	S	22	S	21	I	11	R	0	R	24	S	Positive
2018082847.3-t	0	R	44	S	0	R	30	S	33	S	33	S	24	S	23	S	22	I	10	R	30	S	24	S	Positive
2018081440.2	0	R	24	S	0	R	0	R	29	S	0	R	19	S	21	S	19	I	0	R	0	R	24	S	Positive
2018081440.2-t	0	R	44	S	0	R	27	S	32	S	34	S	24	S	23	S	19	I	0	R	30	S	23	S	Positive
2018092531.2	10	R	36	S	0	R	22	S	29	S	19	S	22	S	14	R	22	I	12	R	24	S	22	S	Positive
2018092531.2-t	0	R	44	S	0	R	28	S	32	S	20	S	28	S	19	I	23	I	11	R	30	S	22	S	Positive
201809183.3	0	R	33	S	0	R	24	S	29	S	25	S	21	S	19	I	18	R	0	R	0	R	17	I	Positive
201809183.3-t	0	R	44	S	0	R	30	S	32	S	33	S	25	S	18	I	20	I	10	R	28	S	21	S	Positive
2018091176.5	0	R	12	R	0	R	25	S	28	S	17	S	20	S	17	R	18	R	10	R	0	R	18	S	Positive
2018091176.5-t	0	R	44	S	0	R	30	S	33	S	34	S	26	S	16	R	18	R	10	R	30	S	21	S	Positive
2018092511.2	0	R	0	R	0	R	22	S	29	S	26	S	20	S	24	S	23	I	0	R	0	R	22	S	Positive
2018092511.2-t	0	R	44	S	0	R	28	S	33	S	33	S	26	S	23	S	22	I	10	R	29	S	22	S	Positive
2018091166.4	0	R	25	S	0	R	0	R	29	S	0	R	20	S	27	S	25	S	12	R	0	R	21	S	Positive
2018091166.4-t	0	R	44	S	0	R	30	S	33	S	35	S	24	S	26	S	26	S	13	R	30	S	22	S	Positive
2018091864.1	0	R	36	S	0	R	0	R	30	S	0	R	12	R	27	S	25	S	14	R	9	R	20	S	Positive
2018091864.1-t	0	R	44	S	0	R	0	R	33	S	34	S	11	R	27	S	26	S	13	R	30	S	20	S	Positive
2018091135.3	0	R	0	R	0	R	0	R	29	S	0	R	0	R	24	S	22	I	9	R	0	R	17	I	Positive
2018091135.3-t	0	R	44	S	0	R	30	S	33	S	34	S	25	S	23	S	21	I	10	R	28	S	22	S	Positive
2018090418.2	0	R	32	S	0	R	24	S	28	S	28	S	20	S	21	S	21	I	0	R	26	S	19	S	Positive
2018090418.2-t	0	R	44	S	0	R	30	S	32	S	33	S	26	S	22	S	21	I	0	R	30	S	23	S	Positive
2018090458.2	0	R	33	S	0	R	24	S	29	S	28	S	22	S	21	S	20	I	0	R	26	S	20	S	Positive
2018090458.2-t	0	R	44	S	0	R	28	S	32	S	35	S	24	S	24	S	22	I	10	R	30	S	23	S	Positive
2018081445.5	0	R	13	R	0	R	0	R	30	S	18	S	20	S	20	I	19	I	11	R	9	R	22	S	Positive
2018081445.5-t	0	R	44	S	0	R	30	S	33	S	32	S	25	S	18	I	24	I	12	R	30	S	22	S	Positive
2018081441.5	0	R	0	R	0	R	0	R	29	S	0	R	10	R	23	S	19	I	10	R	0	R	16	I	Positive
2018081441.5-t	0	R	44	S	0	R	30	S	33	S	35	S	25	S	22	S	20	I	11	R	30	S	22	S	Positive
2018081453.2	0	R	14	R	0	R	0	R	32	S	0	R	14	I	28	S	29	S	18	R	0	R	25	S	Positive
201810092.3	11	R	12	R	0	R	26	S	32	S	0	R	13	I	29	S	29	S	18	R	0	R	26	S	Positive
2018081445.4	0	R	13	R	0	R	0	R	32	S	0	R	13	I	25	S	25	S	15	R	0	R	23	S	Positive
2018102322.3	9	R	14	R	0	R	0	R	28	S	0	R	12	R	26	S	26	S	16	R	0	R	23	S	Positive

t: transconjugant. AMC: amoxicillin-clavulanate; AM: ampicillin; CZ: cefazolin; CAZ: ceftazidime; CTX: cefotaxime; FEP: cefepime; C: chloramphenicol; CIP: ciprofloxacin; GM: gentamicin; IPM: imipenem; TE: tetracycline; SXT: trimethoprim-sulfamethoxazole. R: resistant, I: intermediate, S: susceptible.

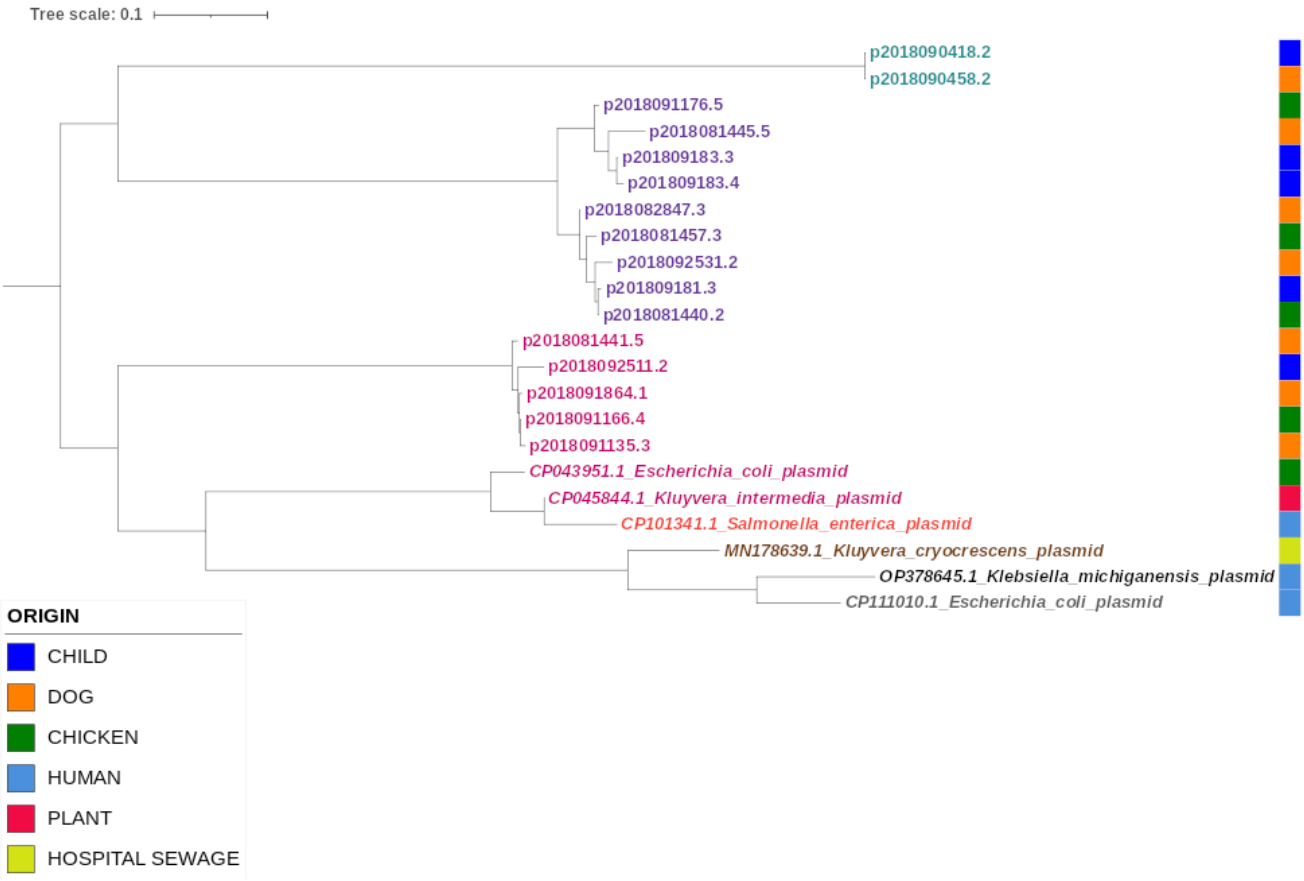

FIG S1. Maximum-likelihood phylogenetic tree of complete sequences of plasmids carrying $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}65}$, and $bla_{CTX\text{-}M\text{-}27}$ allelic variants using closely related plasmid sequences based on BLASTn analyses. The evolutionary history was inferred using GTR tree built using the genetic distance. Labels show plasmid ID assigned based on the host ID followed by its isolate number. The origin of isolate harboring the plasmid is shown by a color strip. Allelic variant held by each plasmid is shown by font colors ($bla_{CTX\text{-}M\text{-}27}$: turquoise; $bla_{CTX\text{-}M\text{-}55}$: purple; $bla_{CTX\text{-}M\text{-}65}$: fuchsia; $bla_{OXA\text{-}1}$: red; $bla_{CTX\text{-}M\text{-}3}$: brown; $bla_{KPC\text{-}3}$: black; $bla_{KPC\text{-}2}$: gray). CP043951.1, isolation year: 2017, location: China. CP045844.1, isolation year: 2017, location: China. CP101341.1, isolation year: 2013, location: China. MN178639.1, isolation year: 2019, location: China. OP378645.1, isolation year: 2018, location: EEUU. CP111010.1, isolation year: 2022, location: China.

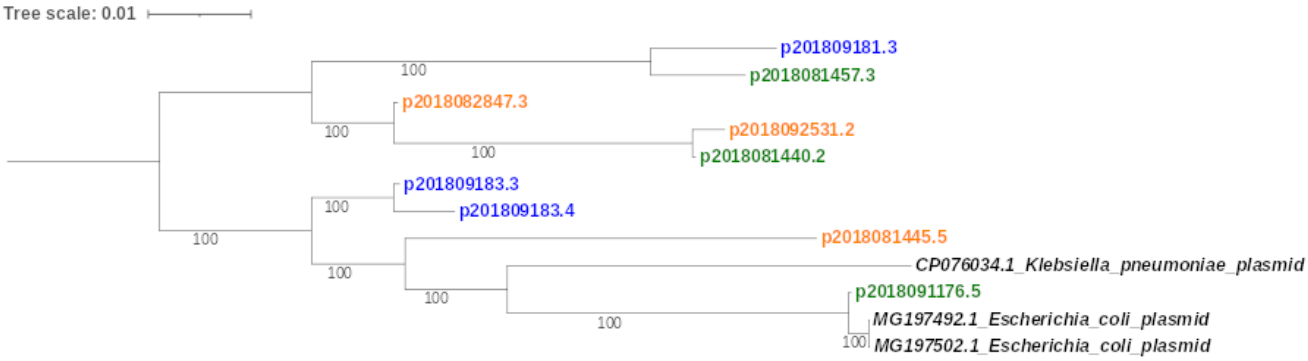

FIG S2. Maximum-likelihood phylogenetic tree of complete sequences of plasmids carrying $bla_{CTX\text{-}M\text{-}55}$ allelic variant using closely related plasmid sequences based on BLASTn analyses. The evolutionary history was inferred using GTR tree built using the genetic distance. Labels show plasmid ID assigned based on the host ID followed by its isolate number. The origin of isolate harboring the plasmid is shown by font colors (child: blue; dog: orange; chicken: green; black: NCBI database). CP076034.1, isolation year: 2022, location: China, allelic variant: $bla_{CTX\text{-}M\text{-}55}$. MG197492.1, isolation year: 2014, location: China, allelic variant: $bla_{CTX\text{-}M\text{-}55}$. MG197502.1, isolation year: 2013, location: China, allelic variant: $bla_{CTX\text{-}M\text{-}55}$. Bootstrap values (> 80) based on 100 replications are shown at the nodes of the tree.

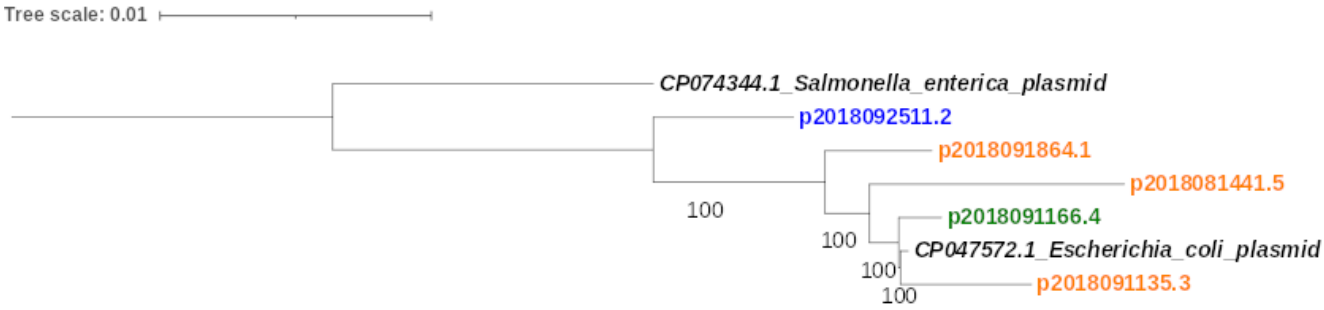

FIG S3. Maximum-likelihood phylogenetic tree of complete sequences of plasmids carrying $bla_{CTX\text{-}M\text{-}65}$ allelic variant using closely related plasmid sequences based on BLASTn analyses. The evolutionary history was inferred using GTR tree built using the genetic distance. Labels show plasmid ID assigned based on the host ID followed by its isolate number. The origin of isolate harboring the plasmid is shown by font colors (child: blue; dog: orange; chicken: green; black: NCBI database). CP074344.1, isolation year: 2010, location: Perú, allelic variant: $bla_{CTX\text{-}M\text{-}65}$. CP047572.1, isolation year: 2010, location: Singapore, allelic variant: $bla_{CTX\text{-}M\text{-}65}$. Bootstrap values (> 80) based on 100 replications are shown at the tree nodes.

Table S2. Number of SNPs and difference length between plasmids that were sequenced in duplicate and plasmids carried by isolates of clonal relationships

	Plasmid ID	Plasmid length (bp)	Difference length (bp)	Difference length (%)	SNPs (nt)	SNPs (%)
Duplicate 1	p201809183.3	71428	89	0.12	41	0.06
	p201809183.3	71339				
Duplicate 2	p2018091864.1	125759	193	0.15	138	0.11
	p2018091864.1	125566				
Duplicate 3	p2018081440.2	94894	16	0.02	23	0.02
	p2018081440.2	94878				
		MEAN		**0.10**		**0.06**
CR - 0 SNPs	p2018090418.2	121366	234	0.19	28	0.02
	p2018090458.2	121132				
CR- 90 SNPs	p2018091135.3	127219	21295	16.74	209	0.20
	p2018081441.5	105924				

CR: clonal relationship (>100 SNPs on core genome).

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CHAPTER 4

General Conclusions

This doctoral research assessed the transmission mechanisms of 3GCR-EC among children and domestic animals in semirural communities of Ecuador through WGS and characterization of MGEs carrying ESBL genes. The genotypic characterization based on pairwise single-nucleotide polymorphisms (SNPs) analysis in the core genome of 3GCR-EC identified clonal relationships (CRs) that involved *E. coli* isolates carrying $bla_{\text{CTX-M}}$ genes associated with either humans or domestic animals, of which several showed evidence of recent transmission. The presence of isolates with CRs and the same allelic variant of $bla_{\text{CTX-M}}$ as well as the genetic similarity of strains among domestic animals and humans, characterized by shared phylogroups and sequence types provide compelling evidence of the shared population of *E. coli* among different host species, suggesting that many *E. coli* lineages are generalists with the ability to colonize the intestines of different animal species.

Additionally, numerous ESBL-encoding $bla_{\text{CTX-M}}$ gene variants were distributed in isolates from humans and domestic animals. The most common allelic variants were $bla_{\text{CTX-M-55}}$ found in similar proportions in isolates from children, dogs and chickens, and $bla_{\text{CTX-M-65}}$ more commonly identified in dog isolates rather than child and chicken isolates. The prevalence of $bla_{\text{CTX-M}}$ allelic variants shared by bacteria in different animal host species was relatively higher than CRs identified among *E. coli* isolates. Therefore, the spread of $bla_{\text{CTX-M}}$ genes in the community could be attributed to clonal transmission and HGT.

The characterization of ESBL-producing *E. coli* performed through long-read sequencing revealed that $bla_{\text{CTX-M}}$ gene was harbored in plasmids and chromosomes. The

backbone structure of plasmids (i.e., replication, maintenance, and plasmid transfer genes) and the synteny were conserved in all plasmids carrying the same *bla*$_{CTX-M}$ allelic variant. All the plasmids and chromosomes carrying *bla*$_{CTX-M}$ allelic variants showed that this gene was bracketed by two IS*26* transposable elements. The IS*26*-*bla*$_{CTX-M}$ bracket (nucleotide sequence containing the *bla*$_{CTX-M}$ gene flanked by two IS*26*s) was the same for all *bla*$_{CTX-M-55}$-carrying plasmids. Conversely, the IS*26*-*bla*$_{CTX-M-65}$ brackets contained the same genes in some plasmids and chromosomes while in others, IS*26*-*bla*$_{CTX-M-65}$ brackets contained fewer genes or genes located in the same order but at different distances from each other.

These findings highlight the critical role of the HGT, particularly of IS*26* transposable element for the current spread and mobility of *bla*$_{CTX-M}$ genes among plasmids or from plasmids to chromosomes, suggesting that nucleotide sequences of IS*26*-*bla*$_{CTX-M}$ brackets could be an important genetic structure to study *bla*$_{CTX-M}$ transmission among humans, domestic animals, and the environment. We provide evidence for the complexity of the *bla*$_{CTX-M}$ HGT and how this understanding can be applied to determine the dissemination of these genes in any community or environment. In addition to those already mentioned, this research underlines the importance of the analysis of the IS*26*-*bla*$_{CTX-M}$ bracket to monitor the transmission of this gene. Understanding the dissemination patterns of AMR genes is critical to implementing effective measures to slow down the dissemination of these clinically important genes.

Although the results establish a strong connection between humans and domestic animals, suggesting that domestic animals and fecal contamination of the household environment by domestic animals likely plays an important role in the transmission of AMR in

the community, it is appropriate to recognize the complexity of transmission dynamics, highlighting the need for further research to determine the transmission directionality (human-to-animal or animal-to-human transmission). Moreover, there could be other routes of exposure to antimicrobial-resistant bacteria and AMR genes, which we have not explore here, such as untreated wastewater or produce irrigated with this water.

Despite these limitations, this study research has enhanced our understanding of complex dynamics of AMR and calls for multifaceted intervention strategies to mitigate its spread across communities and animal species.

In conclusion, the present doctoral research provides strong evidence for overlap of commensal 3GCR-EC strains and ESBL genes, mediated by clonal transmission and HGT, within humans and domestic animals, across relatively large distances in semirural communities of Ecuador.