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Journal

Environmental Health Perspectives, 129(2)

ISSN

1542-4359

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Publication Date

2021-02-01

DOI

10.1289/ehp7729

Peer reviewed

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP7729.

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 3GCR-EC 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 β-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. https://doi.org/10.1289/EHP7729

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; CDC 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-

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Supplemental Material is available online (https://doi.org/10.1289/EHP7729).

The authors declare they have no actual or potential competing financial interests.

Received 22 June 2020; Revised 30 January 2021; Accepted 1 February 2021; Published 22 February 2021.

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 quadrants using satellite imagery, and each quadrant was assigned a random number (using a random numbers table). Households were enrolled in each selected quadrant 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 to select 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 (OPUS) 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). 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 (version 0.12.0).

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 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 y old	6 (20.7)
1 y old	8 (27.6)
2 y old	6 (20.7)
3 y old	7 (24.1)
4 y old	2 (6.9)
Primary caregiver education level	
Elementary	9 (31.0)
High school	15 (51.2)
College	5 (17.2)
Number of 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 water	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 and 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%)
Number of 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)
Number of 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)
Number of 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)
Number of 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)

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 β-D-glucuronidase activity (Lange et al. 2013), followed by the multisubstrate API RapiD-20E identification system (bioMérieux) using a cutoff 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

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 wk			
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 wk			
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 wk			
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.

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%); and *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%); and *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 a 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).

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,

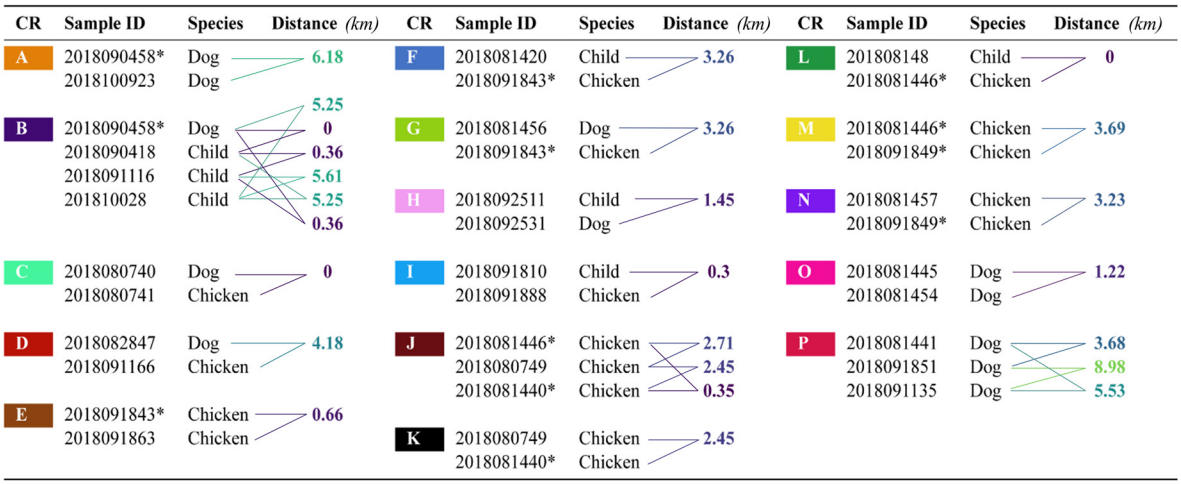

Figure 1. Euclidean distance (in kilometers) between host samples with clonal relationships (CRs) of third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) strains from children, dogs, and chickens. Background colors for each clonal relationship match legend in Figure 2. Longer distances 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.

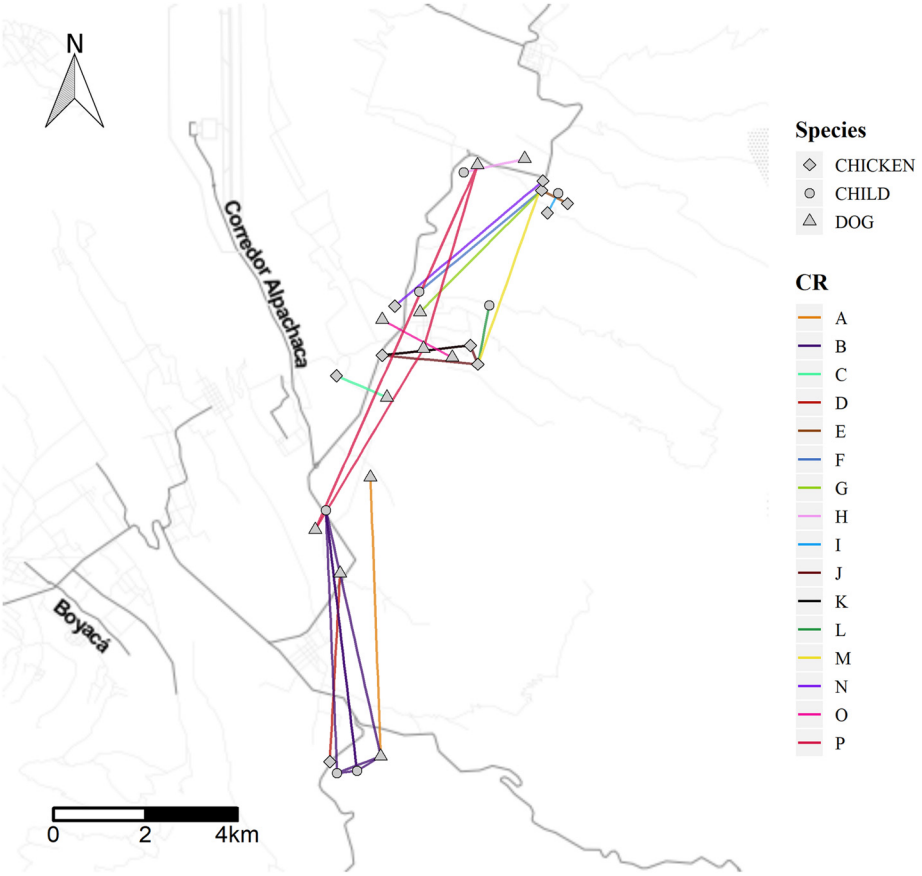

Figure 2. Map of clonal relationships (CRs) among third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) strains in children, dogs, and chickens in peri-urban study site east of Quito, Ecuador.

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).

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 spatio-temporal differences in which other researchers have collected

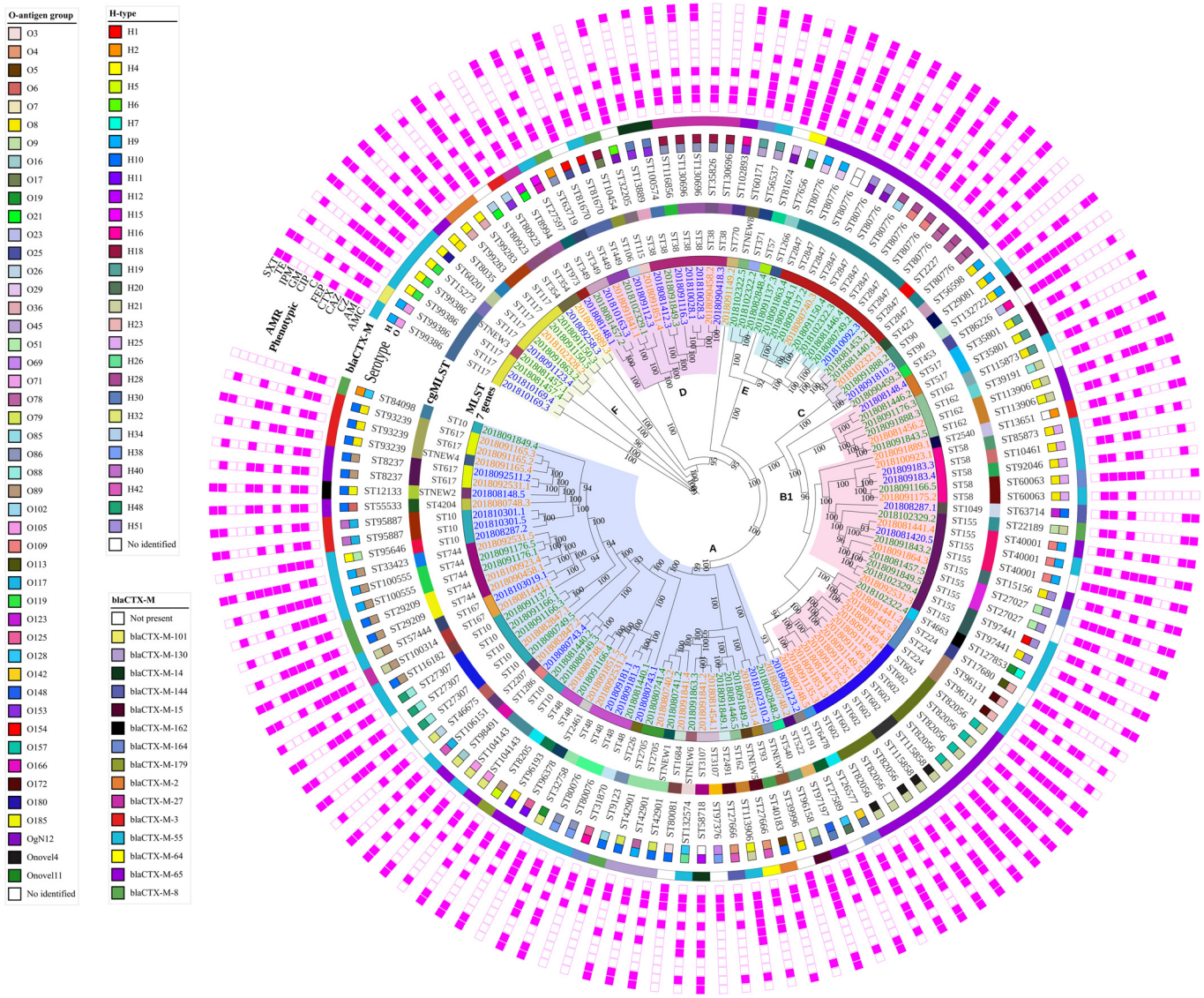

Figure 3. Maximum-likelihood phylogenetic tree of 131 third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolates from children, dogs, and chickens based on core genomes. Labels show isolate ID assigned based on host ID followed by its isolate number. Origin of isolate is shown by 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 $bla_{\text{CTX-M}}$. Pink-colored squares indicate resistance to different antimicrobials. Note: 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.

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,

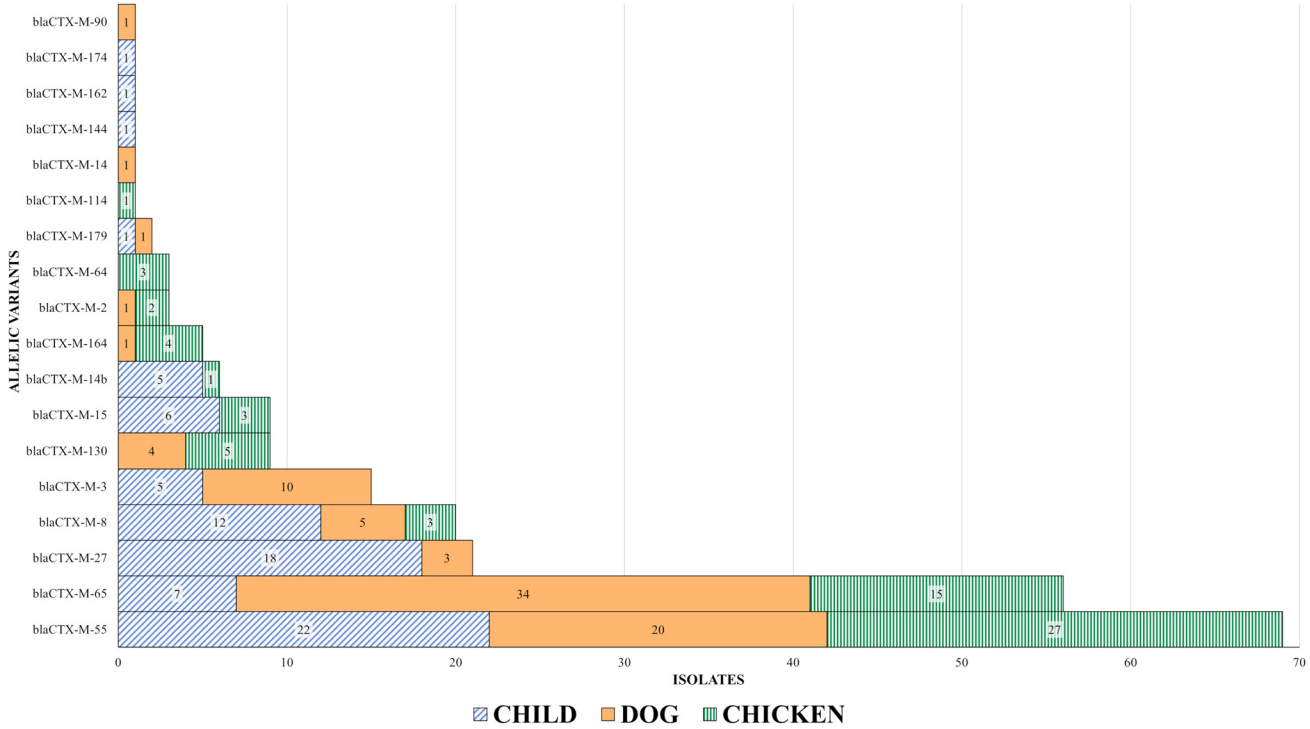

Figure 4. Frequency of allelic variants of $bla_{\text{CTX-M}}$ in third-generation cephalosporin-resistant *Escherichia coli* (3GCR-EC) isolates from children (blue diagonal lines), dogs (orange), and chickens (green vertical lines).

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 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

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the

National Institutes of Health (NIH) under Award Number R01AI135118. P.C. is funded by NIH FIC D43TW010540 Global Health Equity Scholars. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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