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# Impacts of small-scale chicken farming activity on antimicrobial-resistant *Escherichia coli* carriage in backyard chickens and children in rural Ecuador

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## ABSTRACT

The emergence, spread, and persistence of antimicrobial resistance (AMR) remains a pressing global concern. Increased promotion of commercial small-scale agriculture within low-resource settings has facilitated an increased use in antimicrobials as growth promoters globally, creating antimicrobial-resistant animal reservoirs. We conducted a longitudinal field study in rural Ecuador to monitor the AMR of *Escherichia coli* populations from backyard chickens and children at three sample periods with approximately 2-month intervals (February, April, and June 2017). We assessed AMR to 12 antibiotics using generalized linear mixed effects models (GLMM). We also sampled and assessed AMR to the same 12 antibiotics in one-day-old broiler chickens purchased from local vendors. One-day-old broiler chickens showed lower AMR at sample period 1 compared to sample period 2 (for 9 of the 12 antibiotics tested); increases in AMR between sample periods 2 and 3 were minimal. Two months prior to the first sample period (December 2016) there was no broiler farming activity due to a regional collapse followed by a peak in annual farming in February 2017. Between sample periods 1 and 2, we observed significant increases in AMR to 6 of the 12 antibiotics in children and to 4 of the 12 antibiotics in backyard chickens. These findings suggest that the recent increase in farming, and the observed increase of AMR in the one-day old broilers, may have caused the increase in AMR in backyard chickens and children. Small-scale farming dynamics could play an important role in the spread of AMR in low- and middle-income countries.

## 1. Introduction

Small-scale commercial animal farming remains a growing practice throughout the world [1,2]. Poultry is one of the fastest growing meats produced on the planet [3], especially among low- and middle-income countries (LMICs), due to its cost effectiveness and high protein content [4]. Often, small-scale agricultural development programs initiate interventions through the introduction of broiler meat chickens [5,6]. These broilers are typically reared in intensive agricultural farming systems where the chickens harbor high levels of antimicrobial resistance (AMR) and zoonotic pathogens [1,7,8]. There is a current lack of understanding of public health outcomes related to small-scale agriculture since the majority of research focuses on industrial large-scale conventional animal farming systems [7,9]. Industrial large-scale

farming is characterized by raising animals at high densities and using large quantities of chemotherapeutic agents [9]. AMR transmission to human populations from large-scale farming occur primarily through food chain consumption [10], environmental contamination [11], or occupational handling of animals [12]. In contrast, small-scale animal husbandry typically occurs at lower densities within the household setting. These family-operated micro enterprises could potentially promote risk of AMR and zoonosis exposure to community members due to the close proximity of production animals and surrounding human populations [13].

Our study in northwestern coastal Ecuador is an observational investigation, monitoring small-scale broiler farming activity in parallel with human and backyard chicken susceptibility to antimicrobials. In our study communities, small-scale poultry farming of broiler meat

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chickens co-occurs with farming of local backyard chicken. Typically, broiler chickens are reared within a large-scale farming and purchased as one-day-old chickens by farmers whose operations are based out of either a single household or multiple households within a collective hatchery. Our previous analyses suggested that selection for drug resistance in broilers originates in the large-scale farming setting driven by high amounts of antibiotics delivered in the feed [14]. Villagers administer antibiotics supplemented in chicken water source and indirectly through the purchase of commercial chicken feed laced with antibiotics [14].

Intensive farming can have impacts on the emergence of AMR at the farm-level and surrounding community. Our previous work within this study system has demonstrated that compared to backyard chickens, broiler chickens exhibited greater *E. coli* phenotypic antibiotic resistance, greater richness of antimicrobial resistant genes, and lower microbial community diversity [6,14,15]. Elsewhere, conventional farming of poultry compared to antimicrobial use free has been associated with higher AMR prevalence in *Campylobacter* spp. [17]. Multiple studies have noted that vicinity to conventional farming presents increased risk of AMR exposure to human populations [2,16,18–20].

Despite our foundational work, we still have limited understanding of AMR carriage over time in relation to small-scale chicken farming activity. We predict that the introduction of broiler farming activity can function as a driver for changes in AMR profiles over time. Here, we analyzed the variation over time in phenotypic antimicrobial-resistant profiles of backyard chickens and children within rural poultry farming communities in Ecuador.

## 2. Material and methods

### 2.1. Field study design

We conducted our study in three villages within the province of Esmeraldas, Ecuador: Borbón (962 households), Colon Eloy (235 households), and Timbiré (166 households). Between February and May 2017, we collected fecal samples from backyard chickens and children during three observation periods [sample period one (S1): February 2 – February 6, sample period two (S2): March 29 – April 1, and sample period three (S3): May 24 – May 27]. The inclusion criteria for enrollment was that a household had at least one child (age: 5–18) or at least one backyard chicken present. Child samples were provided by parents. Backyard chicken samples were collected by the field team via cloaca chicken swabs.

During each of the three sampling periods, we visited each enrolled household. If at least one child was present, we collected all child fecal samples provided by the household guardians and we administered a survey on antibiotic use of the children living in enrolled households. If a household had backyard chickens, each received a uniquely colored and numbered identification band (National Band & Tag Company) that provided a unique identification number for every backyard chicken enrolled in the study. The field team collected one fecal sample from a maximum of four chickens.

Concurrently, during each observational period we sampled 30 one-day-old broiler chickens from regional vendors as a baseline for understanding the magnitude of potential antibiotic-resistant *E. coli* entering the study villages.

All avian and children samples were placed in Cary Blair medium (Thermo Scientific™) [21] on ice and transported to Quito for analysis within 48 h.

Consent to participate was obtained from all households. The head of a household provided consent on the behalf of the child participants, and provided consent to collect fecal samples from birds. All study protocols were reviewed and approved by the University of Michigan Institutional Review Board (HUM00121496), University of Michigan Institutional Animal Use & Care Committee (PRO00008191), and the Universidad San Francisco de Quito Bioethics Committee (MSP-SDM-

10-2013-1019-O).

### 2.2. Antimicrobial susceptibility testing

We collected field samples and immediately placed them in Cary Blair Transport Medium and cultured within 48 h. In Quito, we plated samples on MacConkey lactose (MKL) agar and then selected up to three lactose positive colonies, which we transferred to a nutrient agar to allow them to grow. Each purified colony was tested for  $\beta$ -glucuronidase activity to identify *E. coli* using Chromocult agar (Merck, Darmstadt, Germany) After confirmation, we again cultured each colony on a nutrient agar for additional growth prior to testing each colony for phenotypic resistance. We used the Kirby-Bauer disc diffusion method [22] for 12 antibiotics: amoxicillin-clavulanate (AMC; 10  $\mu$ g per antibiogram unit), ampicillin (AMP; 10), ciprofloxacin (CIP; 5), cefotaxime (CTX; 30), cephalothin (CF; 30), chloramphenicol (C; 30), enrofloxacin (ENO; 5), gentamicin (GM; 10), streptomycin (S; 10), sulfisoxazole (G; 1 mg), tetracycline (TE; 30), and trimethoprim/sulfamethoxazole (SXT; 25). Zones of inhibition were measured after a 24-h incubation period using digital calipers. Antibiotic sensitivity was recorded for a given child or chicken if at least one of the three isolates was resistant to the antibiotic tested according to Clinical and Laboratory Standards Institute (CLSI) [23,24]. We used reference strains (*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853) as controls for each batch of disc diffusion test. We classified phenotypic resistance as resistant or sensitive (intermediate isolates were categorized as sensitive). Analyses were conducted at the sample level. Multidrug resistance (MDR) was categorized as being resistant to two or more antibiotic classes.

### 2.3. AMR *E. coli* profile turnover

For each sampled child or backyard chicken, we define an AMR profile as the binary categorization of presence or absence of resistance to each of the 12 antibiotics, where we define sample-level resistance as above. For each observational sample period, we estimated the number of profiles by quantifying the number of unique binary categorizations. Profile turnover is defined as the number of unique profiles when comparing one sample period to the next.

### 2.4. Statistical analyses

We used generalized linear mixed effects models (GLMM) to examine changes in phenotypic AMR for individual chickens and children over time. We used sample period as the time variable and included it in the model as a fixed effect. On the other hand, community and individual IDs were included as random effects to account for both community-level clustering and intra-subject correlation, respectively. Conversely, analyses of one-day-old broiler chickens did not include an individual ID. Since multiple chickens might have been acquired from the same vendor, vendor source was included as a random effect to account for vendor-level clustering. Odds ratios and 95% confidence intervals were calculated from GLMM estimates. All analyses were performed using R Statistical Software version 3.5.2 (2019).

## 3. Results

### 3.1. Chicken-farming activities before and during study period

During our three sampling periods, the mean number of households raising broiler chickens ranged from 2 to 7 in Borbón, 1 to 3 in Colon Eloy and 0 to 1 in Timbiré (Fig. 1). Overall, the mean number of broilers chickens reared per farm was 58 (min-max: 0–132). There was no farming activity in December 2016. By February 2017 there was a mean of 114 chickens per farming household. Anecdotally, our field supervisor reported that the collapse in December 2016 in broiler farming

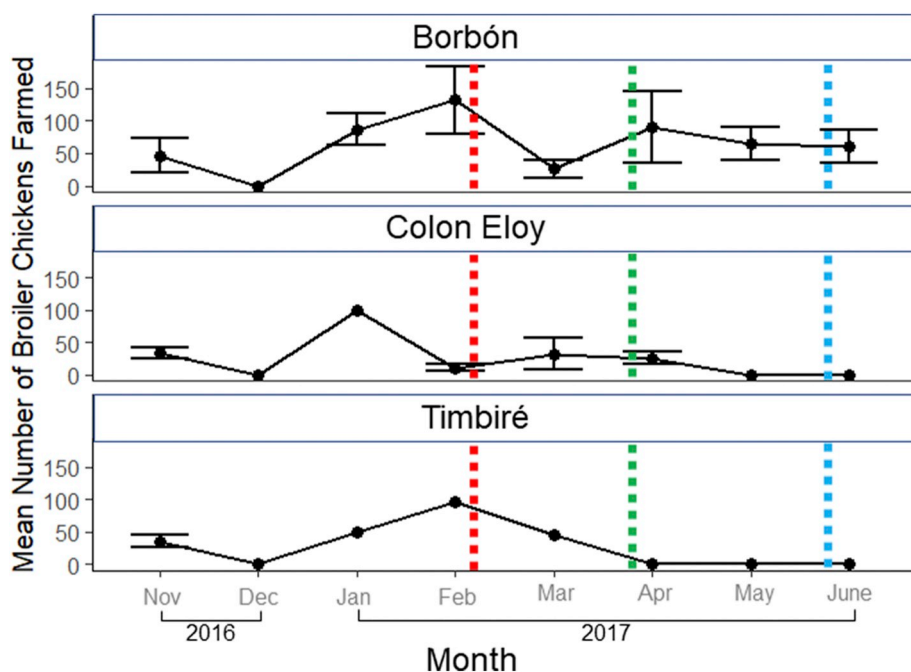


Fig. 1. 2016–2017 monthly mean ( $\pm$  SE) number of broiler chicken farmed in three villages within the Esmeraldas Province, Ecuador. Standard error was calculated by the square root of the number of houses actively farming (points without SE bars indicate  $n$  households  $\leq 1$ ). Colors correspond to dates of sample periods (red: sample period one, green: sample period two, blue sample period three). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was due to an earthquake in the region leading to high broiler mortality. We enrolled 70 households (34 from Borbón, 25 from Colon Eloy and 11 from Timbiré). Of these households, 57 had backyard chickens and at least one child, 13 had only backyard chickens.

### 3.2. AMR in one-day-old broiler chicken from vender sources

Multiple drug resistance (MDR) of one-day-old broiler chickens (resistance to two or more unique antibiotic classes) was 68.4%, 100% and 100% for each observational period S1, S2, and S3 respectively (Table 1). Across the three observational periods, the mean number of

Table 1

Phenotypic resistance profiles of *E. coli* isolates collected from one-day-old vendor source broiler chickens from three regional poultry vendors located in Borbón, San Lorenzo, and Esmeraldas City in Esmeraldas Province, Ecuador (Borbón, San Lorenzo during sample periods one (S1), two (S2), and three (S3). A GLMM was used to compare S1 vs. S2 and S2 vs. S3. A significant difference between S2 and S1 ( $P$ -value  $< .05$ ) is represented by the symbol \* in column 2. For the comparison between S2 and S3, the symbol is located in column 3. Each cell contains the number of antibiotic-resistant *E. coli* isolates and the percentage resistant of those tested,  $n$ . MDR, multiple drug resistance, is defined as resistance to two or more unique antibiotic classes.

Antibiotic	S1	S2	S3
	( $n = 76$ )	( $n = 87$ )	( $n = 90$ )
MDR	52 (68.4)	87 (100)	90 (100)
Amoxicillin-clavulanate	22 (28.9)	45 (51.7)*	15 (16.7)*
Ampicillin	18 (23.7)	69 (79.3)	83 (92.2)
Cefotaxime	2 (2.6)	60 (69.0)*	65 (72.2)
Cephalothin	54 (71.1)	79 (90.8)*	82 (91.1)
Chloramphenicol	13 (17.1)	76 (87.4)*	63 (70.0)
Ciprofloxacin	2 (2.6)	57 (65.5)*	42 (46.7)
Enrofloxacin	3 (3.9)	56 (64.4)*	60 (66.7)
Gentamicin	1 (3.7)	10 (11.5)*	17 (18.9)
Streptomycin	37 (46.7)	82 (94.3)*	75 (83.3)
Sulfisoxazole	23 (30.3)	79 (90.8)*	82 (91.1)
Tetracycline	47 (61.8)	87 (100)	90 (100)
Trimethoprim/Sulfamethoxazole	23 (30.3)	79 (90.8)*	82 (91.1)

antibiotics that a given isolate was resistant to was 7, with 64% of the isolates resistant to 7 or more antibiotics. We recorded the resistance to “AMP-G-AMC-CTX-CF-C-CIP-SXT-ENO-TE-S” ( $n = 13$ ) as the most frequently observed resistance profile for one-day-old broiler chickens also present in one child and two backyard chickens. Comparing S1 and S2, we detected an increase in AMR for 10 of the 12 antibiotics tested (Table 1). The mean number of antibiotics an isolate was resistant to, increased from 3 (S1) to 9 (S2). In general, resistance levels for S3 was similar to that of S2. Resistance prevalence to 5 of the 12 antibiotics tested were all above 90% during S2 [cephalothin (CF), sulfisoxazole (G), tetracycline (TE), streptomycin (S), and trimethoprim/sulfamethoxazole (SXT)] and S3 [ampicillin (AMP), cephalothin (CF), sulfisoxazole (G), tetracycline (TE), and trimethoprim/sulfamethoxazole (SXT)]. Comparing S2 and S3, we observed a statistically significant decline in resistance to only amoxicillin-clavulanate (AMC).

### 3.3. Change in AMR in children and backyard chickens over time

We detected high *E. coli* phenotypic resistance. For example, MDR ranged from 76.1 to 88.3% in children and 81.1 to 83.3% in backyard chickens (Table 2). The gain in the proportion of children isolates with AMR between sample S1 and S2 varied from 13.7% (GM) to 45.1% (SXT) (Supplementary Materials; Table 1), while gains in backyard chickens isolates varied from 8.7% (S) to 52.2% (SXT) (Supplementary Materials; Table 2). Specifically, we detected increased AMR in children samples ( $n = 220$ ) for GM, S, AMP, CTX, G, and SXT comparing S1 to S2 (Table 3; GLMM,  $p < .05$ ). Among samples from backyard chickens ( $n = 96$ ), we detected increased AMR levels from S1 to S2 for GM, CIP, ENO, and SXT (Table 3, Table 4; GLMM,  $p < .05$ ). From S2 to S3, we detected decreased AMR levels in children for AMC, C, and MDR, and in backyard chicken for AMC, CF and MDR. This result remained constant when including additional covariates including flock size, binary categorization of broiler chickens farmed, and total number of farming durations during the study period.

Among children and backyard chickens there were 103 AMR profiles identified in S1, 114 in S2, and 100 in S3 (Table 5). We observed a high amount of unique *E. coli* AMR profiles in each sample period: 69 for S1, 86 for S2, and 69 for S3. At the individual level, we collected as many as 3 isolates. Comparing S1 to S2, we observed that 77% of the samples had an AMR profile in all 3 isolates, 18.9% of the samples had

**Table 2**

Phenotypic resistance prevalence of *E. coli* isolates collected from backyard chickens and children during sample periods one (S1), two (S2), and three (S3). Each cell contains the number of antibiotic-resistant *E. coli* isolates and the percentage resistant of those tested, *n*. MDR, multiple drug resistance is defined as two or more unique antibiotic classes.

Antibiotic	Child			Backyard chicken		
	S1	S2	S3	S1	S2	S3
	( <i>n</i> = 72)	( <i>n</i> = 77)	( <i>n</i> = 71)	( <i>n</i> = 30)	( <i>n</i> = 37)	( <i>n</i> = 29)
MDR	56 (77.8)	68 (88.3)	54 (76.1)	25 (83.3)	30 (81.1)	21 (81.1)
Amoxicillin-clavulanate	72 (31.9)	24 (31.1)	12 (16.9)	18 (60.0)	13 (35.1)	2 (6.9)
Ampicillin	72 (51.4)	51 (66.2)	41 (57.7)	13 (43.3)	19 (51.4)	16 (55.2)
Cefotaxime	8 (11.1)	19 (24.7)	17 (23.9)	7 (23.3)	10 (27.0)	3 (10.3)
Cephalothin	52 (72.2)	66 (85.7)	57 (80.3)	25 (83.3)	25 (67.6)	17 (58.6)
Chloramphenicol	17 (23.6)	15 (19.5)	12 (16.9)	6 (20.0)	12 (32.4)	6 (20.7)
Ciprofloxacin	8 (11.1)	18 (23.4)	8 (11.3)	1 (3.3)	5 (13.5)	2 (6.9)
Enrofloxacin	11 (15.3)	19 (24.7)	14 (19.7)	3 (10.0)	8 (21.6)	5 (17.2)
Gentamicin	6 (8.3)	62 (19.5)	9 (12.7)	2 (6.7)	10 (27.0)	6 (20.7)
Streptomycin	72 (84.7)	74 (96.1)	59 (83.1)	27 (90.0)	33 (89.2)	26 (89.7)
Sulfisoxazole	26 (36.1)	45 (58.4)	36 (50.7)	10 (33.3)	20 (54.1)	14 (48.3)
Tetracycline	49 (68.1)	58 (75.3)	49 (69.0)	19 (63.3)	25 (67.6)	20 (69.0)
Trimethoprim/ Sulfamethoxazole	18 (25.0)	42 (54.5)	35 (49.3)	7 (23.3)	20 (54.1)	12 (41.4)

**Table 3**

Odds ratio and 95% CI comparing *E. coli* sample phenotypic resistance to 12 antibiotics among children samples comparing sample period one (S1) to two (S2) and S2 and three (S3).

Antibiotic	S1 vs S2		S2 vs S3	
	OR	95% CI	OR	95% CI
Amoxicillin-clavulanate	0.7	0.4 – 1.2	0.7	0.2 – 1.6
Ampicillin	2.3	1.4 – 4.0	0.5	0.3 – 2.0
Cefotaxime	2.9	1.4 – 5.9	0.5	0.2 – 3.6
Cephalothin	2.1	1.0 – 4.2	0.4	0.2 – 2.2
Chloramphenicol	1.3	1.3 – 1.3	0.5	0.2 – 1.7
Ciprofloxacin	2.1	1.0 – 4.3	0.2	0.1 – 1.8
Enrofloxacin	1.9	0.9 – 3.7	0.4	0.1 – 2.2
Gentamicin	2.7	1.2 – 6.0	1.9	0.1 – 3.6
Streptomycin	2.9	1.1 – 7.7	0.1	0.1 – 1.5
Sulfisoxazole	2.2	1.3 – 3.8	0.5	0.2 – 2.0
Tetracycline	1.1	0.6 – 1.9	0.7	0.2 – 1.9
Trimethoprim/ Sulfamethoxazole	2.7	1.5 – 4.7	0.4	0.2 – 2.0

**Table 4**

Odds ratio and 95% CI comparing *E. coli* sample phenotypic resistance to 12 antibiotics among backyard chicken samples comparing sample period one (S1) to two (S2) and sample period two (S2) to three (S3).

Antibiotic	S1 vs S2		S2 vs S3	
	OR	95% CI	OR	95% CI
Amoxicillin-clavulanate	0.7	0.4 – 1.2	0.6	0.1 – 2.8
Ampicillin	1.0	1.0 – 1.5	1.0	0.4 – 2.5
Cefotaxime	1.8	1.0 – 1.5	0.3	0.1 – 1.4
Cephalothin	1.2	0.7 – 2.1	0.5	0.1 – 1.2
Chloramphenicol	2.3	1.0 – 3.2	0.3	0.1 – 1.5
Ciprofloxacin	231.1	6.3 – 784.7	0.0	0.0 – 57.9
Enrofloxacin	7.3	1.4 – 36.9	0.0	0.0 – 6.6
Gentamicin	2.7	1.5 – 5.4	0.5	0.2 – 2.8
Streptomycin	1.5	0.8 – 3.1	0.6	0.2 – 2.8
Sulfisoxazole	1.3	0.8 – 2.0	0.9	0.4 – 2.3
Tetracycline	1.1	0.7 – 1.8	1.0	0.4 – 2.8
Trimethoprim/ Sulfamethoxazole	1.8	1.1 – 2.8	0.6	0.3 – 2.0

an AMR profile turnover in 2 of the 3 isolates, and 4.1% of the samples had an AMR profile turnover in 1 of the 3 isolates. We did not observe any samples that retained the same profile when comparing S1 and S2.

**Table 5**

Summary of AMR *E. coli* profiles (binary categorizations, either resistance or susceptible, of the 12 antibiotics tested) detected among children and backyard chicken isolates.

	S1	S2	S3
No. of isolates tested	103	114	100
Backyard Chickens	31	37	29
Children	72	77	71
No. of unique profiles identified (%)	69 (67)	86 (75)	69 (69)
Backyard Chickens	23 (74)	34 (92)	24 (83)
Children	46 (64)	52 (68)	45 (63)
No. of new profiles relative to prior sample period (%)			
Backyard Chickens	–	28 (82)	23 (96)
Children	–	38 (73)	29 (64)

In sum, 92% and 68% of the isolates in S2 were new profiles in backyard chickens and children respectively. We observed new profiles when comparing S2 to S3 in both backyard chickens (96%) and children (64%) (Table 5).

#### 4. Discussion

The emergence, spread, and persistence of antibiotic resistance remains a pressing concern especially among LMICs where the practice of small-scale animal husbandry is common [6,25–27]. Our observed increase in *E. coli* resistance among backyard chickens and children could have been in response to a resurgence in farming activity in our Ecuadorian study system. Increases in the resistance levels of one-day-old broiler chickens originating from the large-scale farm could have also contributed to the observed resistant patterns. Our additional observation of a high rate of profile turnover when comparing isolates between S1 and S2, suggests either the introduction of new *E. coli* strains and/or high levels of horizontal gene transfer. Together, these results suggest that AMR can readily spillover from farmed chickens to backyard chickens and ultimately to children.

In December 2016, we observed a collapse in small-scale broiler farming activity followed by a large peak in February 2017. A earthquake within the region. This magnitude of mortality is not surprising, since meat broiler chickens are vulnerable to various environmental pressures, including natural disasters [28], infectious diseases [29], and temperature change [30]. All of these drivers of avian mortality are likely intensified within tropical environments [31,32].

We suspect that any observed increase in phenotypic resistance levels in backyard chickens due to the increase in farming intensity is likely delayed given that it takes time for the drug-resistant microbes and genes to spread into the environment. To ensure we captured the response to the increase in farming we set our sampling period to 60 days. Previous work has recorded AMR bacteria and genes persisting in animal feces and soil for up to 60 days [33,34], suggesting that 60 days was a reasonable choice.

We speculate that antibiotic use in broiler farming is an important driver for AMR increases in children and backyard chickens. Our previous studies have all reported limited to no antibiotic use in backyard chickens and significantly higher levels of AMR in broiler chickens [6,14,15]. In addition, while antibiotic use in humans within our study system is significant [35], based on our data on child antibiotic use prior to each sampling period, there was not a significant increase in use patterns that would explain the increases we observed in AMR.

Previous longitudinal studies within industrial food animal agricultural systems have demonstrated that decreases in antibiotic use in farming practices can decrease AMR levels. An 18-month study documented a decline in swine MRSA carriage following a 44% decline in antimicrobial use [36]. From 2011 to 2013, loss in extended-spectrum beta-lactamase-producing *E. coli* was associated with the absence of cephalosporin use in swine farming [37]. The impact of the European Union ban on antibiotic use as growth promoters in farming, however, has had mixed results. In Denmark, resistance to vancomycin among certain bacterial species appeared to persist following the ban on growth promoters [38].

Our study is distinct in showing the impact of a recent resurgence of farming intensity on AMR. Interestingly, only for trimethoprim-sulfamethoxazole did we detect a gain in resistance to both children and backyard chickens. We reported a similar finding in our prior work, which is likely due the physical link between a mobile genetic element (*int1*) and trimethoprim-sulfamethoxazole and the observation that *int1* is strongly associated with farming activity [15]. It is surprising that we observed gains in ciprofloxacin resistance from backyard chicken samples because ciprofloxacin is a wide spectrum clinically relevant quinolone that is seldom used in humans within our study site [35].

The other main source of antibiotics are the one-day-old chickens coming from the large-scale farms, which constantly change the antibiotics applied to the chickens prior to being sold to farmers. As a result, the resistant profiles of the one-day-old broiler chickens also varied over time. The high turnover rate of resistance profiles in *E. coli* may reflect the frequent exposure to novel *E. coli* strains in the environment driven by broiler farming and/or may reflect a high rate of horizontal gene transfer. Other studies have documented how resistant strains of commensal bacteria can outcompete non-resistant species leading to overall lower microbiota diversity [17,39]. Our previous studies have detected decreases in broiler *E. coli* phenotypic resistance levels over time, largely due to the absence of selection pressure from the large-scale farm setting [6]. Analogously, within the same studied villages, we reported greater microbial diversity and lower abundance in antimicrobial-resistant genes sampled from backyard chickens compared to broiler chickens [15].

## 5. Conclusion

Small-scale chicken farming is an important economic activity in our study communities. It is also an important source of antibiotic resistance. The specific implications of this source of AMR is complex. Future studies should focus on disentangling these ecological complexities. In this regard, longitudinal studies can be useful. Following newborns to monitor long-term patterns of AMR in humans, chickens and the environment can provide important data on determinants of the spread of AMR. Other important data include knowledge of antimicrobials and concentrations delivered in feed to understand the dynamic selection pressures within this system, and bacterial strain

sequencing to measure the rate of genetic exchange between animals and humans [40]. These focused inquiries can help us more deeply understanding the dynamics of antibiotic susceptibility in animal and human populations.

## Declaration of Competing Interest

The authors do not have any conflict of interest regarding this research.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2019.100112>.

## References

- [1] N.V. Cuong, et al., Antimicrobial usage in animal production: a review of the literature with a focus on low- and middle-income countries, *Antibiotics*. 7 (3) (2018) 1–20.
- [2] J. Carrique-Mas, et al., Mortality, disease and associated antimicrobial use in commercial smallscale chicken flocks in the Mekong Delta of Vietnam, *Prev. Vet. Med.* 165 (2019) 15–22.
- [3] J.J. Dibner, J.D. Richards, Antibiotic growth promoters in agriculture: history and mode of action, *Poult. Sci.* 84 (4) (2005) 634–643.
- [4] D. Tilman, et al., Global food demand and the sustainable intensification of agriculture, *Proc. Nat. Acad. Sci.* 108 (50) (2011) 20260–20264.
- [5] M. Gilbert, et al., Income disparities and the global distribution of intensively farmed chicken and pigs, *PLoS One* 10 (7) (2015) 1–14.
- [6] H.D. Hedman, et al., High prevalence of extended-spectrum beta-lactamase CTX-M-producing *Escherichia coli* in small-scale poultry farming in rural Ecuador, *Amer. J. Trop. Med. Hyg.* 100 (2) (2019) 374–376.
- [7] J.P. Graham, et al., Hyperendemic *Campylobacter jejuni* in Guinea pigs (*Cavia porcellus*) raised for food in a semi-rural community of Quito, Ecuador, *Environ. Microb. Report.* 8 (3) (2016) 382–387.
- [8] K. Vasco, et al., Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in Ecuador, *Appl. Environ. Microb.* 82 (14) (2016) 4218–4224.
- [9] M.J. Gilchrist, et al., The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance, *Environ. Health Persp.* 115 (2) (2007) 313–316.
- [10] C. Verraes, et al., Antimicrobial resistance in the food chain: a review, *Int. J. Environ. Res. Pub. Health* 10 (7) (2013) 2643–2669.
- [11] W.Y. Xie, et al., Antibiotics and antibiotic resistance from animal manures to soil: a review, *euro, J. Soil Sci.* 69 (7) (2017) 181–195.
- [12] N. Trung, et al., Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam, *J. Antimicrob. Chemother.* 70 (7) (2015) 2144–2152.
- [13] J.P. Graham, et al., Small-scale food animal production and antimicrobial resistance: mountain, molehill, or something in-between? *Environ. Health Perspect.* 125 (10) (2017) 104501.
- [14] N.P. Braykov, et al., Antibiotic resistance in animal and environmental samples associated with small-scale poultry farming in northwestern Ecuador, *mSphere*. 1

- (1) (2016) 1–15.
- [15] X. Guo, et al., Antibiotic resistome associated with small-scale poultry production in rural Ecuador, *Environ.Sci.Tech.* 52 (15) (2018) 8165–8172.
- [16] K.A. Moser, et al., The role of mobile genetic elements in the spread of antimicrobial-resistant *Escherichia coli* from chickens to humans in small-scale production poultry operations in rural Ecuador, *Amer. J. Epid.* 187 (3) (2017) 558–567.
- [17] T. Luangtongkum, et al., Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry, *App. Environ. Microb.* 72 (5) (2006) 3600–3607.
- [18] C. Jernberg, et al., Long-term ecological impacts of antibiotic administration on the human intestinal microbiota, *ISME J.* 1 (1) (2007) 56–66.
- [19] N. Trung, et al., Zoonotic transmission of *mcr-1* colistin resistance gene from small-scale poultry farms, Vietnam, *Emerg. Infect. Dis.* 23 (3) (2017) 529–532.
- [20] Y., Yamamoto, et al., Colistin-resistant *Escherichia coli* with *mcr* genes in the livestock of rural small-scale farms in Ecuador, *BMC Res. Notes.* 12 (2019) 121.
- [21] D.A. Neumann, et al., Cary-Blair, a transport medium for *Vibrio parahaemolyticus*, *Am. J. Clin. Pathol.* 57 (1972) 33–34.
- [22] J.J. Biemer, Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method, *Institute Clin. Sci.* 74 (2) (1973) 511–519.
- [23] Clinical and Laboratory Standards Institute, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, Approved Standard M31-A4, 28 Clinical and Laboratory Standards Institute, Wayne, PA, 2009, p. 47.
- [24] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; 22nd Informational Supplement. CLSI, Clinical and Laboratory Standards Institute, Wayne, PA, 2012, pp. M100–S22.
- [25] L.A. Andoh, et al., Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry in Ghana, *Epidemiol. Infect.* 144 (15) (2016) 3288–3299.
- [26] E.O., Ngbebe, et al., Antimicrobial resistance and virulence profile of enterococci isolated from poultry and cattle sources in Nigeria, *Trop. Anim. Health Prod.* 49 (3) (2017) 451–458.
- [27] T.K.N. Bui, et al., Potential transmission opportunity of CTX-M-producing *Escherichia coli* on a large-scale chicken farm in Vietnam, *J. Global Antimicrob. Res.* 13 (2018) 1–6.
- [28] S. Glassey, T. Wilson, Animal welfare impact following the 4 September 2010 Canterbury (Darfield) earthquake, *Australas. J. Disaster & Trauma Stud.* (2011) 49–59.
- [29] A. Agunos, et al., Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada, *Can. Vet. J.* 53 (12) (2012) 1289–1300.
- [30] C.W. Ritz, et al., Evaluation of hot weather thermal environment and incidence of mortality associated with broiler live haul, *Poult. Sci. Assoc.* 14 (3) (2005) 594–602.
- [31] I. Henry, et al., Prevalence and risk factors for campylobacter spp. in chicken broiler flocks in Reunion Island (Indian Ocean), *Prev. Vet. Med.* 100 (1) (2011) 64–70.
- [32] C. Tirawattanawanich, et al., The effects of tropical environmental conditions on the stress and immune responses of commercial broilers, Thai indigenous chickens, and crossbred chickens, *Appl. Poult. Res.* 20 (4) (2011) 409–420.
- [33] H. Heuer, K. Smalla, Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months, *Environ. Microbiol.* 9 (3) (2007) 657–666.
- [34] A. Scott, et al., Enrichment of antibiotic resistance genes in soil receiving composts derived from swine manure, yard wastes, or food wastes, and evidence for multi-year persistence of swine *Clostridium* spp, *Can. J. Microbiol.* 64 (3) (2018) 201–208.
- [35] J.N.S. Eisenberg, et al., In-roads to the spread of antibiotic resistance: regional patterns of microbial transmission in northern coastal Ecuador, *J. R. Soc. Interface* 45 (7) (2012) 1029–1039.
- [36] A. Dorado-García, et al., Dose-response relationship between antimicrobial drugs and livestock-associated MRSA in pig farming, *Emerg. Infect. Dis.* 21 (6) (2015) 950–959.
- [37] W. Dohmen, et al., Risk factors for ESBL-producing *Escherichia coli* on pig farms: a longitudinal study in the context of reduced use of antimicrobials, *PLoS One* 12 (3) (2017) 1–14.
- [38] F.M. Aarestrup, et al., Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark, *Antimicrob. Agen. Chemoth.* 45 (7) (2001) 2054–2059.
- [39] J. Lin, et al., Response of intestinal microbiota to antibiotic growth promoters in chickens, *Food Pathogens. Dis.* 10 (4) (2013) 331–337.
- [40] L. Armand-Lefevre, et al., Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs, *Emerg. Infect. Dis.* 11 (5) (2005) 711–714.