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High Prevalence of Extended-Spectrum Beta-Lactamase CTX-M–Producing *Escherichia coli* in Small-Scale Poultry Farming in Rural Ecuador

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Abstract. Small-scale farming may have large impacts on the selection and spread of antimicrobial resistance to humans. We conducted an observational study to evaluate antibiotic-resistant *Escherichia coli* populations from poultry and humans in rural northwestern Esmeraldas, Ecuador. Our study site is a remote region with historically low resistance levels of third-generation antibiotics such cefotaxime (CTX), a clinically relevant antibiotic, in both poultry and humans. Our study revealed 1) high CTX resistance (66.1%) in farmed broiler chickens, 2) an increase in CTX resistance over time in backyard chicken not fed antibiotics (2.3–17.9%), and 3) identical $bla_{\text{CTX-M}}$ sequences from human and chicken bacteria, suggesting a spillover event. These findings provide evidence that small-scale meat production operations have direct impacts on the spread and selection of clinically important antibiotics among underdeveloped settings.

Small-scale agriculture is a growing practice throughout the world.[1] It is estimated that more than 80% of the chicken population worldwide occurs in small-scale food systems, yielding up to 90% of the total poultry products in many low- and middle-income countries (LMICs).[2] Antimicrobial agents are commonly administered in small-scale agricultural settings to maximize animal growth and survival,[3] resulting in the potential for antimicrobial resistant (AMR) bacteria to spillover into other animals and humans. We examine this avian-to-human spillover potential in rural communities in northern coastal Ecuador.

To date, most of the work studying the transmission pathway of agricultural animal-to-human transmission of AMR focuses on concentrated animal feeding operations CAFOs[3,4] and bacteria isolated under antibiotic pressure (media enriched with antibiotics). These large-scale food production facilities are defined by their high density of food production animals with high levels of subtherapeutic antibiotic use for growth promotion.[5] Human exposure can occur through occupational handling or consumption of poultry products.[4] By contrast, small-scale agricultural operations raise fewer animals, but often at the household setting, resulting in high risk for human exposure. Because of this proximity of humans to livestock, small-scale agriculture has the potential for animal-to-human spillover events.

Our present study in northwestern Ecuador is a model system for understanding the spread of antibiotic resistance in an underdeveloped, agricultural setting. In rural villages of Esmeraldas Province, Ecuador, small-scale poultry farming of broiler chickens co-occurs with farming of local backyard chicken breeds. Typically, broiler chickens are commercially bred within a CAFO and purchased as chicks by small-scale farming operations that either are based out of a single household or run by multiple households in a shared coop. These chickens have been exposed to high levels of antibiotics both at the CAFO and while being raised in the communities. Our prior analysis suggests that the CAFO environment compared with the community is the major driver of the antibiotic resistance observed in broiler chickens.[5] By contrast, backyard chickens are a local variety of chicken that are free grazing around the household in the open environment and seldom prescribed chemotherapeutic agents. Foundational work from this study region has demonstrated higher phenotypic antibiotic resistance levels[5] and higher levels of mobile genetic elements[6] in broiler chickens compared with backyard chickens.

Between June and July 2015, we sampled chickens from households raising both broiler and backyard breeds within a community in the province of Esmeraldas, Ecuador. Specifically, our study design comprises two observational periods monitoring antibiotic resistance among chickens in 10 households at the time that the household received 30 broiler chickens to farm and 1 month later. We also sampled backyard chickens from 10 households in a control community (CC) where there was no broiler chicken farming (Table 1). In the farming community (FC), we collected a maximum of 10 samples from backyard chickens residing in the 10 households during both observation periods. If a household had fewer than 10 chickens, we sampled all backyard chickens. On the other hand, we sampled 10 broiler chickens from only two of the 10 households. We chose two households because of the close proximity of shared animal husbandry environment. In the CC, we sampled 10 households to fulfill a maximum of 10 backyard chickens but only during the first sample period. Antibiotic resistance data from children were collected 2 years later between February and May 2017 from the same FC that received broiler chickens. The chicken samples were collected via cloacal swabs, and children samples were provided by their parent guardian. All samples were placed in Cary Blair media and transported to Quito for analysis. Consent to participate was obtained from all households, and all study protocols were reviewed and approved by the University of Michigan Institutional Review Board and the Universidad San Francisco de Quito Bioethics Committee.

Bacteria were grown on selective media; *Escherichia coli* isolates were selected and analyzed for resistance to 12

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TABLE 1

Phenotypic resistance profiles for backyard chickens (sampled from the baseline and farming community [FC]) and broiler chickens both collected during sample periods one (S1) and two (S2)

		Backyard chickens		Broiler chickens	
Antibiotic	Control community, S1 (n = 143)	FC, S1 (n = 127)	FC, S2 (n = 195)	FC, S1 (n = 59)	FC, S2 (n = 60)
Gentamicin	0 (0)	7 (5.5)	12 (6.1)	30 (50.8)	7 (11.8)
Streptomycin	20 (13.9)	65 (51.1)	42 (21.6)	57 (96.6)	42 (72.4)
Amoxicillin–clavulanate	1 (0.7)	0 (0)	5 (2.5)	12 (20.3)	6 (10.0)
Ampicillin	11 (7.6)	40 (31.4)	38 (19.4)	52 (88.1)	24 (40.0)
Cefotaxime	2 (1.3)	3 (2.3)	35 (17.9)	39 (66.1)	44 (73.3)
Cephalothin	43 (30.0)	36 (28.3)	56 (28.7)	47 (79.6)	24 (40.0)
Chloramphenicol	14 (9.7)	24 (18.8)	38 (19.4)	13 (22.0)	27 (45.0)
Sulfisoxazole	26 (20.8)	57 (44.8)	64 (35.7)	51 (86.4)	41 (74.5)
Ciprofloxacin	2 (1.3)	6 (4.7)	14 (7.1)	31 (52.5)	12 (20.0)
Enrofloxacin	2 (1.4)	8 (6.4)	3 (5.7)	24 (40.6)	13 (25.4)
Trimethoprim/sulfamethoxazole	14 (9.7)	47 (37.0)	36 (18.4)	51 (86.4)	30 (50.8)
Tetracycline	53 (37.0)	73 (57.4)	93 (48.1)	56 (94.9)	42 (76.3)

Each cell contains the number of antibiotic-resistant *Escherichia coli* isolates and the percentage resistant of those tested, n.

antibiotics through Kirby Baur disc diffusion as described in detail in prior publications.[5,6] We classified phenotypic resistance as resistant or sensitive (intermediate isolates were categorized as sensitive) and tested for differences between sample periods one and two using generalized linear mixed-effects models to control for repeated observations at the household level (Table 2). For all isolates that had phenotypic resistance to cefotaxime (CTX), we screened for the presence of the bla_{CTX} gene and sequenced with Sanger sequencing. A phylogenetic tree was generated via maximum likelihood analysis in MEGA version 7.0 software (www.megasoftware.net).[6]

During the first sampling period, S1, backyard chickens had statistically lower phenotypic resistance levels than broiler chickens for all antibiotics tested except for chloramphenicol and tetracycline (Table 2). Cefotaxime, a clinically relevant third-generation cephalosporin, was the only antibiotic that significantly increased in the backyard chickens between S1 and S2 at 2.3% and 17.9%, respectively (Table 1).

When comparing the backyard chickens in the FC with the neighboring CC, we observed higher levels of phenotypic resistance in the FC for ampicillin, streptomycin, trimethoprim/sulfamethoxazole, ampicillin, and sulfisoxazole. We speculate that these higher levels of phenotypic resistance among backyard chickens are due to previous farming activity within the FC before the onset of this study.

When comparing the broiler chicken phenotypic resistance levels between the two sampling periods, we observed a statistically significant decline in streptomycin, trimethoprim/sulfamethoxazole, and sulfisoxazole (Table 2). This decline in phenotypic resistance followed the findings of Braykov et al.[5] and is likely due to the high levels of antibiotics received at the CAFO while in the egg and shortly after hatching; after these chicks are purchased and moved to the community household setting, these resistance levels subsequently declined over time.

In our genetic analysis, we detected a substantially higher presence of $bla_{CTX\text{-}M}$, a common gene associated with CTX resistance, in broiler chickens than in backyard chickens for both sample periods S1 (82.9% [n = 39] versus 0.0% [n = 0]) and S2 (68.8% [n = 31] versus 22.9% [n = 8]). Phylogenetic analysis clustered $bla_{CTX\text{-}M}$ genes into two clades, suggesting a shared evolutionary history among our chicken and human samples.

The intensive use of antibiotics in small-scale agriculture from LMICs is on the rise and has potential to develop and spread AMR to human populations. We observed initial high levels of CTX phenotypic resistance in broilers followed by fade out in several antibiotics; although broiler chickens received supplementary antibiotics via commercial feed, our data suggest that the greater selection pressure for resistance occurs within a CAFO setting.[5] On the other hand, at the household level we detected a meaningful rise in CTX phenotypic resistance among backyard

TABLE 2

Odds ratio and 95% CI comparing phenotypic resistance to 12 antibiotics among four comparison groups (P-value < 0.05*)

	FC S1 (backyard chicken)		FC S2 (backyard chicken)		FC S2 (broiler chicken)		FC S1 (broiler chicken)	
	CC S1 (backyard chicken)		FC S1 (backyard chicken)		FC S1 (broiler chicken)		FC S1 (backyard chicken)	
Antibiotic tested	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Gentamicin	–	–	0.42	0.17–1.02	2.39	0.98–5.83	8.01	1.05–61.24*
Streptomycin	4.51	1.39–14.65*	0.30	0.13–0.71	3.34	1.42–7.87*	23.84	3.19–178.37*
Amoxicillin–clavulanate	1.50	0.14–16.06	0.76	0.25–2.38	1.31	0.42–4.06	18.80	5.02–70.46*
Ampicillin	5.05	1.43–17.83*	0.57	0.26–1.26	1.75	0.80–3.83	9.49	1.91–47.13*
Cefotaxime	1.08	0.06–20.56	25.65	3.56–185.0*	0.04	0.01–0.28	1,124	14.62–86,440.20*
Cephalothin	1.02	0.34–3.06	0.71	0.32–1.54	1.41	0.75–3.88	10.72	2.09–55.07*
Chloramphenicol	1.89	0.57–6.31	1.74	0.82–3.69	0.57	0.27–1.22	3.20	0.78–13.10
Sulfisoxazole	4.56	1.43–14.59*	0.39	0.17–0.91	2.54	1.01–5.87*	9.21	1.56–54.46*
Ciprofloxacin	4.77	0.99–23.02	0.59	0.26–1.34	1.70	0.75–3.88	7.55	3.28–17.37*
Enrofloxacin	4.82	0.99–23.58	0.50	0.19–1.35	2.00	0.74–5.34	7.95	3.05–20.73*
Trimethoprim/sulfamethoxazole	7.59	1.83–31.42*	0.29	0.12–0.72	3.41	1.39–8.33*	19.34	2.28–164.32*
Tetracycline	3.00	0.99–8.79	0.48	0.20–1.17	2.09	0.85–5.12	4.52	0.99–20.49

CC = control community; CI = confidence interval; FC = farming community; OR = odds ratio. We were unable to run a statistical model for gentamicin to compare backyard chickens of the CC with the FC because there were no phenotypic resistant isolates.

chickens (with no direct exposure to antibiotics) 1 month after the introduction of broiler chickens. Furthermore, sequenced $bla_{CTX\text{-}M}$ from our human and backyard chicken bacteria sources exhibited a shared evolutionary history embedded with broiler chickens, demonstrating that broiler chickens have greater $bla_{CTX\text{-}M}$ diversity than humans.

Cefotaxime extended-spectrum beta-lactamases (ESBLs) have globally emerged as the most common type of ESBL.[7] In our study, CTX resistance was isolated without using antibiotic-selective plates, suggesting that these isolates were present at significant numbers and were not a minority strain. In our study region, however, CTX resistance in *E. coli* has been very low in the past decade and has not exceeded beyond approximately 0.5% among human isolates.[6] We therefore speculate that this shared source of CTX resistance entry into backyard chicken and human populations originated from broiler chickens. These broiler chickens came from a regional producer of chickens that consistently administers a variety of antibiotics, including cephalosporins, to hens and eggs before selling to farmers.[5]

Most food-producing animals have exhibited higher resistance when comparing backyard and nonconventional animals.[1,4–6,8–12] Our detection of a potential spillover event through the rise in CTX phenotypic resistance in backyard chickens (Table 1) expands on these studies. Typically, LMIC settings have poor sanitation infrastructure that can promote spillover because of the spread of these bacteria through the environment, and our data suggest this spread can occur even with no direct contact between backyard and broiler chickens.

Antibiotic use remains a pressing concern in LMICs, where small-scale intensive animal production farming is on the rise.[1,7,13,14] In Ecuador, one study found that nearly half of the producers considered the use of antibiotics important for growth promotion, especially when animals are young.[15] This intensive use of antibiotics has many implications, including greater AMR gene richness and lower taxonomic diversity compared with backyard chickens.[14]

Small-scale introductions of intensively raised food animals over a short duration may yield lasting effects on the surrounding environment. Development projects promoting these small-scale farms could inadvertently promote these spillover events of organisms and genes encoding resistance to antibiotics of clinical importance. Further longitudinal data and analysis is necessary to understand the effect that intensively farmed poultry introductions may have on other animals, humans, and the overall resistome.

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