

Important foodborne bacteria and their antimicrobial resistances in Ecuadorian poultry

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Introduction

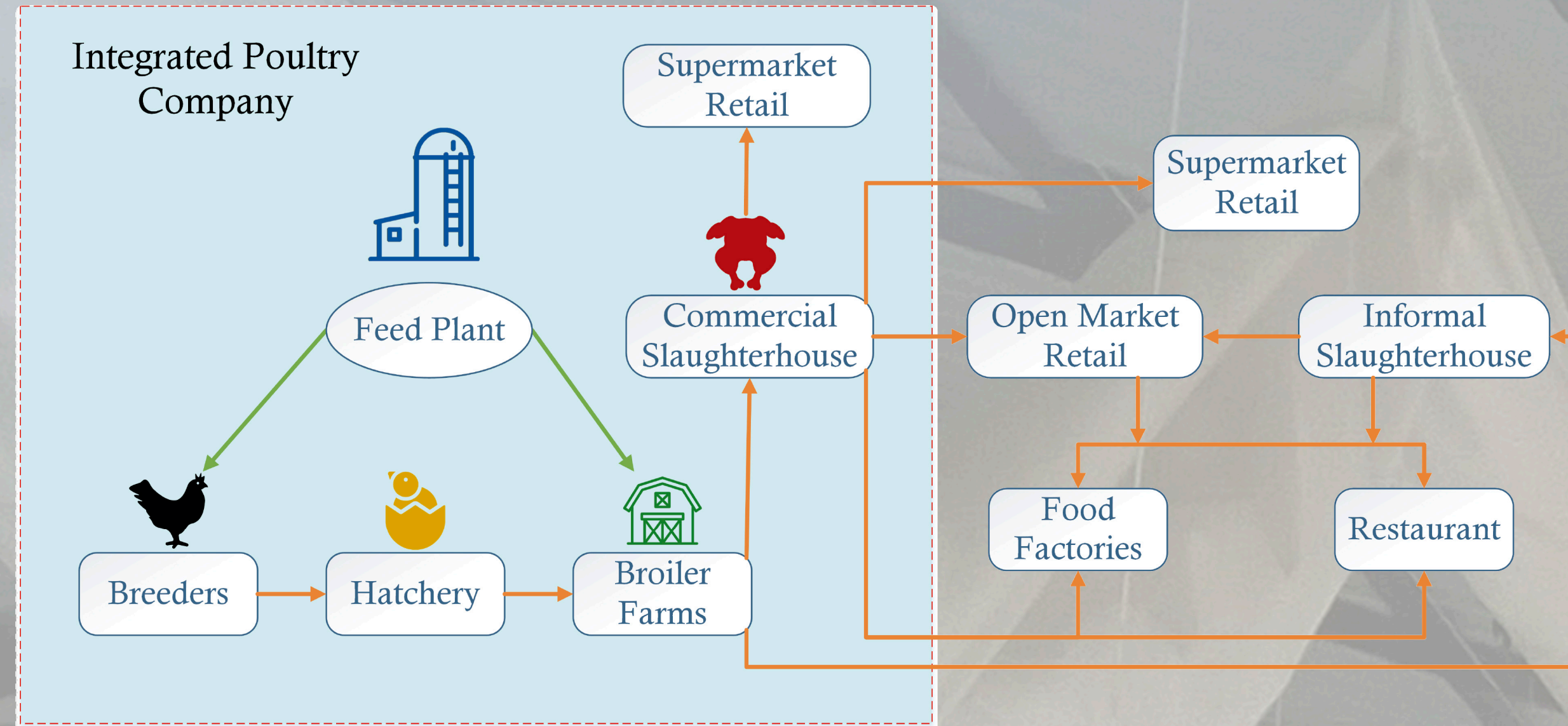
Foodborne bacteria and their antimicrobial resistances are a major health concern in the world. In many tropical countries, this issue has been partially addressed because the lack of funds, public policies and technical limitations. In Ecuador, poultry meat is the main source of animal protein for human consumption.

Aim

We aimed to study the prevalence and antimicrobial resistance of *E. coli* ESBL, *Salmonella* and *Campylobacter* from the poultry industry in Ecuador.

Methods

388 broiler flocks coming from 120 farms were sampled during one year. Bacteriological isolation of *E. coli* ESBL, *Salmonella* and *Campylobacter* were carried out. Isolates were further typed with molecular techniques and antimicrobial resistant profiles were accessed by phenotypic (EUCAST ECOFFs) and genotypic methods (ESBL Genes).



Ecuadorian poultry production is carried out in integrated poultry companies.



Yellow circle denotes sampling area.

Results

Campylobacter

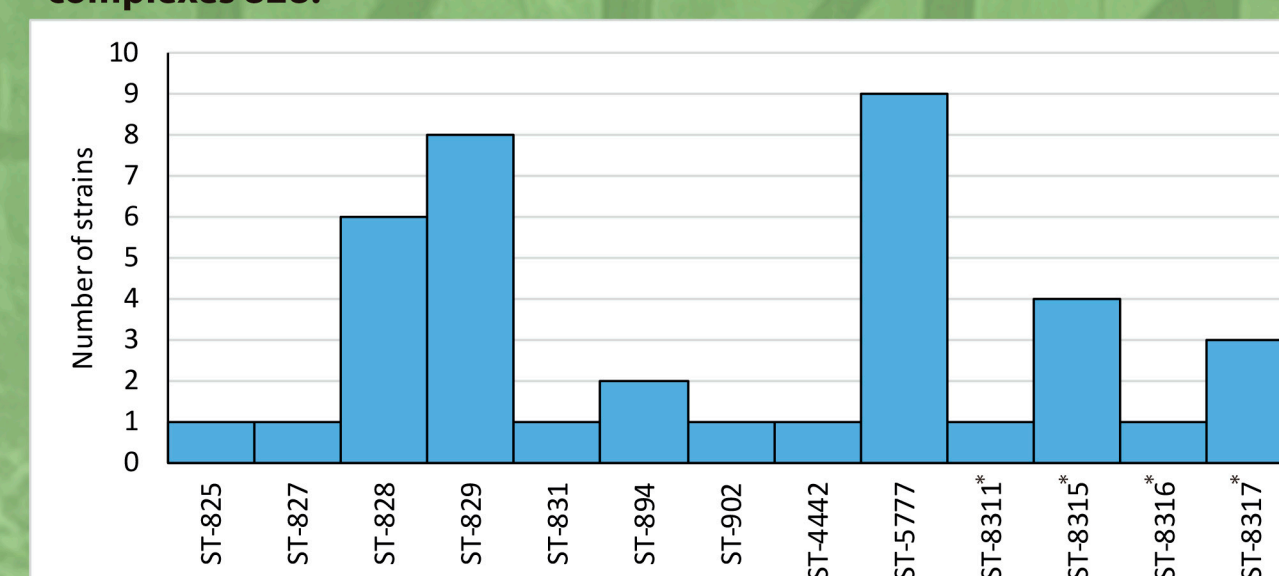
Campylobacter was present in 64% of broiler flocks. *Campylobacter coli* was mostly isolated (64.1%), while *Campylobacter jejuni* was present in 18.9% of samples. 12.4% of isolates were positive for *C. coli* and *C. jejuni*. *Campylobacter* isolates showed high resistant phenotypes against most tested antibiotics. Genetic typing showed that isolates from different farms were closely related.

Antibiotic resistance patterns of *C. coli* and *C. jejuni* isolates

Pattern	Resistance pattern ¹	<i>C. coli</i> (%)	<i>C. jejuni</i> (%)
1	GSCTEN	2 (1.2)	1 (2.1)
2	SCTEN	14 (8.2)	1 (2.1)
3	SCTN	3 (1.8)	2 (4.2)
4	CTEN	23 (13.5)	0
5	CTN	73 (42.9)	35 (72.9)
6	CEN	5 (2.9)	0
7	TN	0	1 (2.1)
8	CN	49 (28.8)	8 (16.7)
9	C	1 (0.6)	0
Total		170 (100)	48 (100)

¹Gentamicin (G), ciprofloxacin (C), nalidixic acid (N), tetracycline (T), streptomycin (S), erythromycin (E).

Distribution of STs among the 49 *C. coli* strains belonging to clonal complexes 828.



* Novel STs identified in *Campylobacter* strains

Distribution of the minimal inhibitory concentration values for 48 *C. jejuni* isolates collected from broiler batches.

Antibiotic	Number of <i>C. jejuni</i> isolates with minimal inhibitory concentrations (µg/µl) ¹										
	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Gentamicin	42	3	2	1							
Streptomycin	2	5	35	2	2	2					
Erythromycin	23	21	2								1
Tetracycline	6	2		1	1	28					10
Ciprofloxacin	1				2	33	3	9			
Nalidixic acid							1	1			46

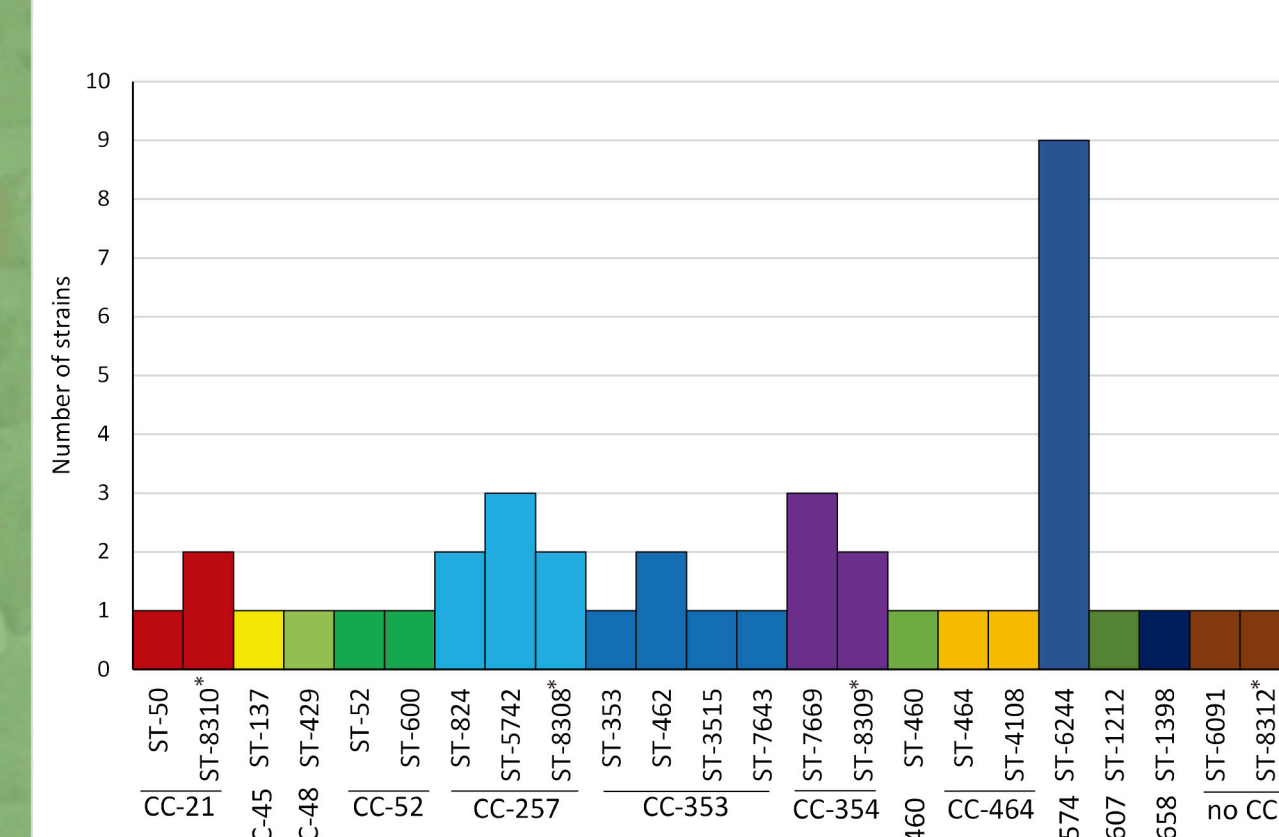
¹Full vertical lines indicate epidemiological break points for resistance described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

Distribution of the minimal inhibitory concentration values for 170 *C. coli* isolates collected from broiler batches.

Antibiotic	Number of <i>C. coli</i> isolates with minimal inhibitory concentrations (µg/µl) ¹										
	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Gentamicin	106	49	8	5	1						
Streptomycin	1	120	30					19			
Erythromycin	38	33	33	22	6				31		7
Tetracycline	5	34	15	6		1	1	89			24
Ciprofloxacin		1			2	134	5	28			
Nalidixic acid							1		1	11	157

¹Full vertical lines indicate epidemiological break points for resistance described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015).

Distribution of STs and clonal complexes among the 40 *C. jejuni* strains.



* Novel STs identified in *Campylobacter* strains

Salmonella

Salmonella was present in 16% of flocks. *S. Infantis* was the most isolated serotype (83.9%) followed by *S. Enteritidis* (14.5%) and *S. Corvallis* (1.6%). *Salmonella* isolates showed high resistant phenotypes against most tested antibiotics.

Distribution of the minimal inhibitory concentration values for the 62 *Salmonella* isolates collected from the positive broiler batches

Antibiotic	Number of <i>Salmonella</i> isolates with minimal inhibitory concentrations (µg/µl)											Resistance %					
	0.02	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Sulfamethoxazole																	
Gentamicin																	
Ciprofloxacin	5	2	3														
Ampicillin																	
Cefotaxime	8	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ceftazidime																	
Tetracycline																	
Streptomycin																	
Trimethoprim																	
Chloramphenicol																	
Colistin																	
Floxacillin																	
Kanamycin																	
Nalidixic acid																	

Full vertical lines indicate epidemiological break points for resistance described by European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015). Clinical break points for resistance described by the Clinical and Laboratory Standards Institute (CLSI, 2014) were used for Kanamycin and Sulfamethoxazole.

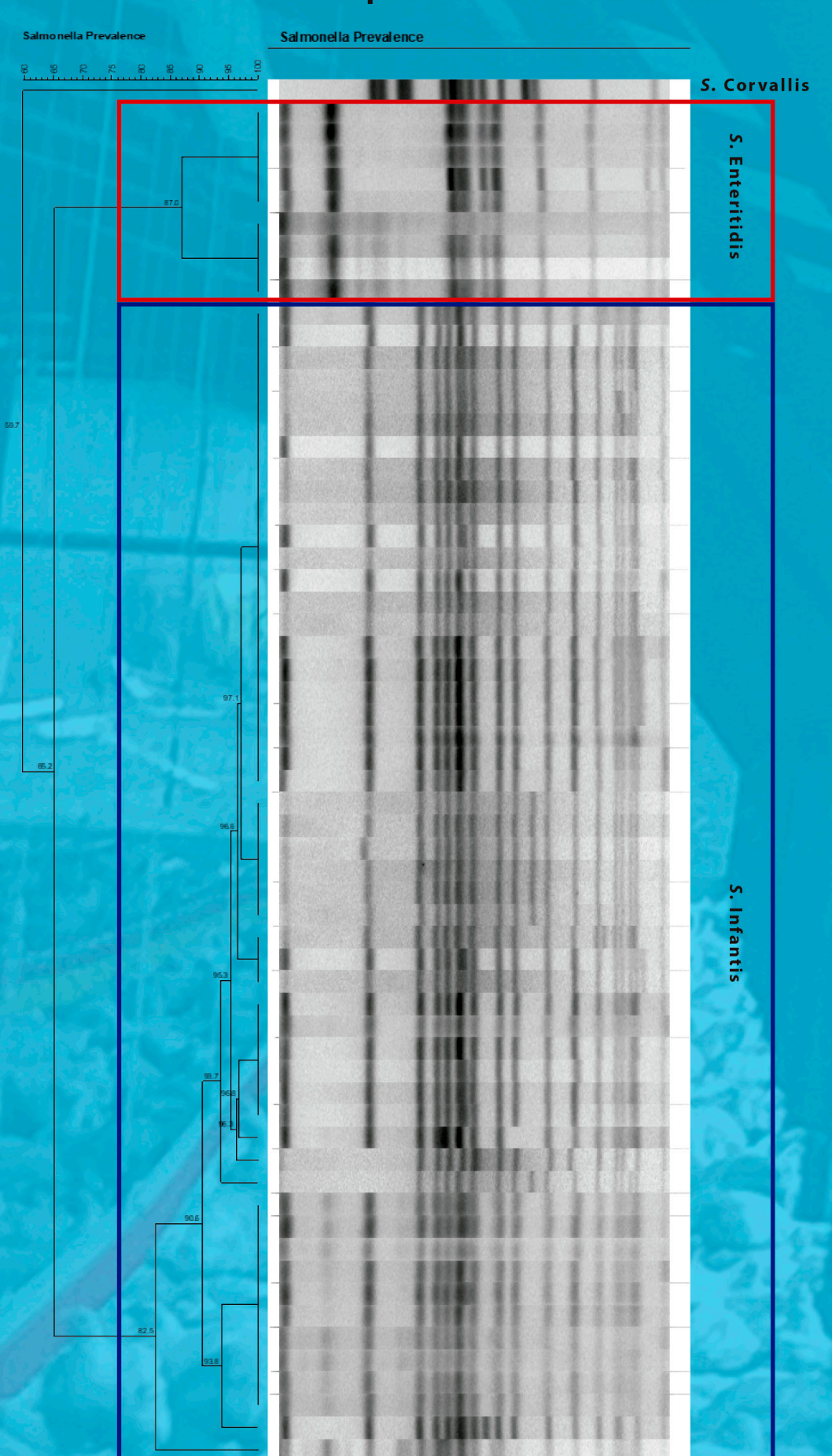
Antibiotic resistance patterns of *Salmonella* strains and phenotypes of cefotaxime resistant strains

Pattern	Resistance pattern	No. Antibiotics	S. Infantis	S. Enteritidis	S. Corvallis	Rate (%)	ESBL+ strains*	blaCTX+ strains*	AmpC+ strains*
1	SGCAFZTRMHNKL	13	2			3.2%	2	2	
2	SGCAFRMHNKL	12	20			32.3%	15	15	5
3	SGCAFRMHNKL	12	1			1.6%			1
4	SGCAFRMHNKL	12		1		1.6%			1
5	SGCFZRMHNKL	12		1		1.6%			1
6	SGCAFRMHNKL	11	6			9.7%	6	5	
7	SGCAFRMHNKL	11	2			3.2%	2	2	
8	SGAFTRMHNKL	10	1			1.6%	1	1	
9	SGCAFRMHNKL	10	1			1.6%	1	1	
10	SGCAFRMHNKL	10	1			1.6%	1	1	
11	SGCAFRMHNKL	10	1			1.6%	1	1	
12	SGCFZRMHNKL	10	3			4.8%	NA	NA	NA
13	SCAFTRMNL	9	1			1.6%			1
14	GCAFTRMHNKL	9	1			1.6%	1	1	
15	SCFRMHNKL	8	1			1.6%	NA	NA	NA
16	SCAFZTRL	8	1			1.6%	1	1	
17	SCAFTRL	7	3			4.8%	3	3	
18	SCFRML	7	1			1.6%			1
19	SCFRML	6	4			6.5%	NA	NA	NA
20	STRML	5	1			1.6%	NA	NA	NA
21	SCM	3	2			1.6%	NA	NA	NA
22	SO	3	1			1.6%	NA	NA	NA
23	SM	2	1			1.6%	NA	NA	NA
24	O	1		6		9.7%	NA	NA	NA
Total			52	9	1		34	33	10

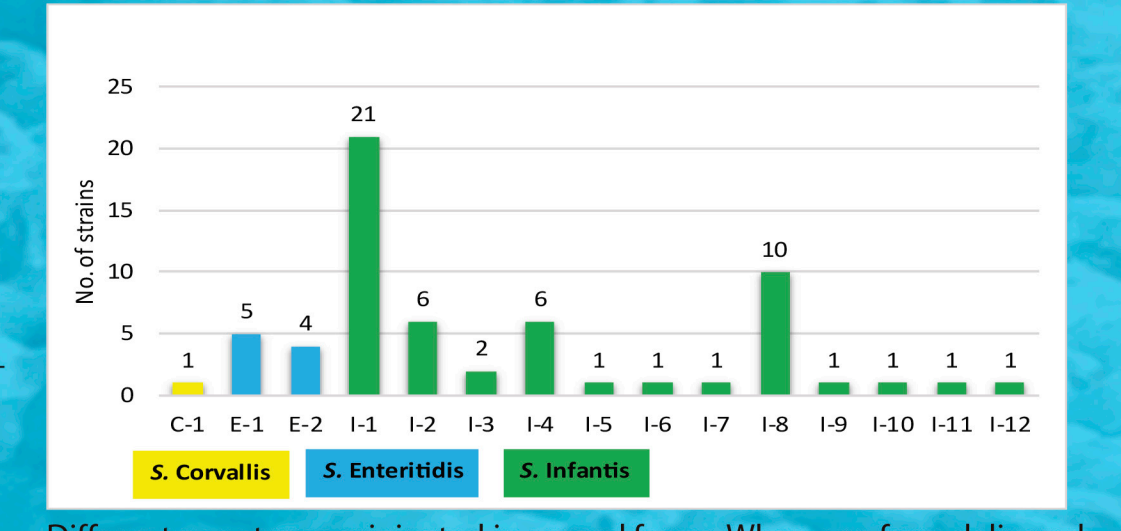
Sulfamethoxazole (S), ciprofloxacin (C), nalidixic acid (L), tetracycline (T), trimethoprim (M), cefotaxime (F), ampicillin (A), florfenicol (N), gentamicin (G), chloramphenicol (H), kanamycin (K), streptomycin (R), colistin (O) and ceftazidime (Z). NA: Not Applicable.

*Number of strains with ESBL or AmpC phenotype according to disk diffusion test.

PFGE profiles of the 62 *Salmonella* isolates collected from the positive broiler batches.



Salmonella genotypes present in each serotype



Different genotypes originated in several farms. When one farm delivered two or more positive batches different genotypes/serotypes were found.

E. coli ESBL

E. coli ESBL, was present in 92% of broiler flocks. *E. coli* ESBL harboured genes of the families blaCTX-M (90,6%), blaSHV (10%), blaTEM (42%) and blaCMY (22%). The gene mcr-1 responsible for resistance to colistin was found in 3 isolates. Resistance genes kpc and mcr-2 were not found. *E. coli* showed high resistant phenotypes against most tested antibiotics. Genetic typing showed that isolates from different farms were closely related.

Antibiotic resistance patterns of *E. coli* ESBL isolates

Pattern ¹	Groups of antibio.	No. of isolates	Pattern ¹	Groups of antibio.	No. of isolates
XBTAPFQ	7	22	BTAQ	4	2
BTAPFQ	6	4	BTQ	4	3
XBAPFQ	6	3	TAFQ	4	5
XBTAFQ	6	4	TAPQ	4	1
XBTAPQ	6	3	XAPQ	4	1
XTAPFQ	6	20	XBPQ	4	1
BAPFQ	5	2	XTAQ	4	2
BTAQ	5	5	XTAFQ	4	1
BTAPQ	5	1	XTPQ	4	1
BTPFQ	5	1	AFQ	3	1
TAPFQ	5	1	TAF	3	1
XBAPF	5	2	TAQ	3	1
XBTAP	5	1	TFQ	3	1
XBTAQ	5	2	XAP	3	1
XTAFQ	5	3	XTP	3	2
XTAPQ	5	5	FQ	2	2
XTPFQ	5	2	TQ	2	1
BAPQ	4	1	Q	1	1

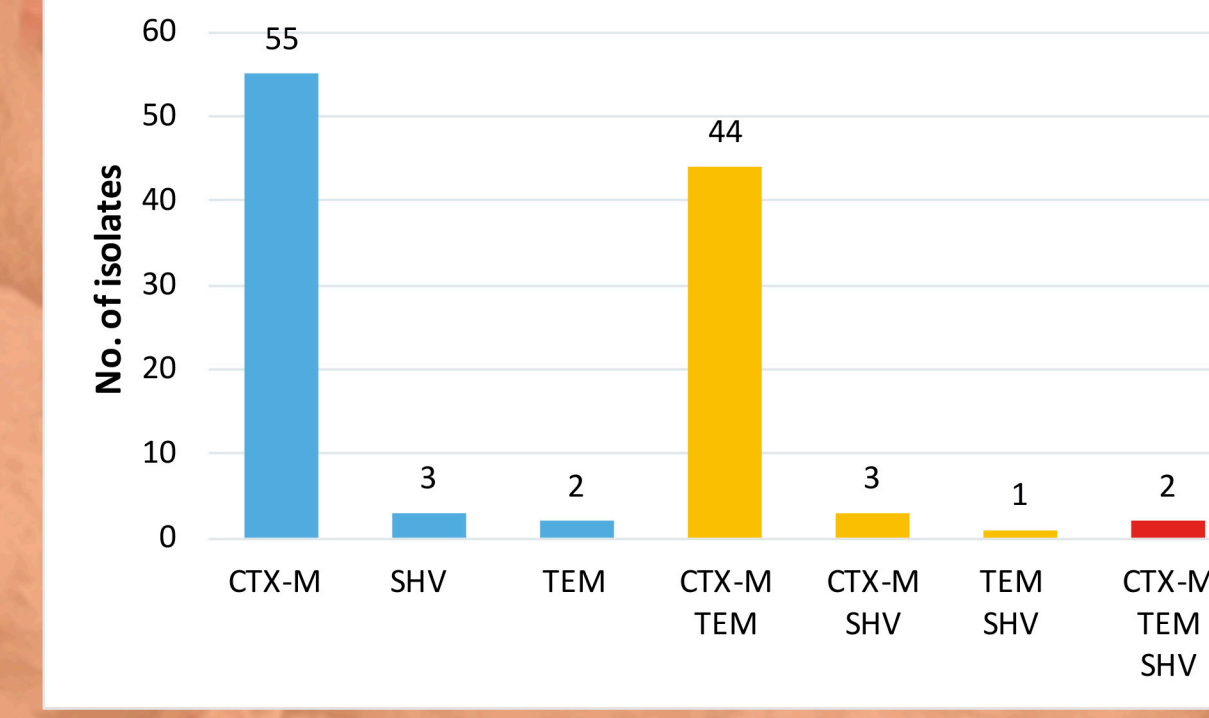
¹Folate Pathway Inhibitors (X), Tetracycline (T), Aminoglycoside (A), Phenolics (P), Nitrofurans (N), Quinolones (Q), Betalactamic (B).

Antibiotic resistance patterns of *E. coli* AmpC isolates

Pattern ¹	Groups of antibio.	No. of isolates
XBTAPFQ	7	25
BTAPFQ	6	5
XBAPFQ	6	3
XBTAFQ	6	8
XBTAPF	6	2
XBTAPQ	6	5
XBTPFQ	6	2
XTAPFQ	6	1
BAPFQ	5	2
BTAQ	5	2
BTPFQ	5	1
XBAPF	5	1
XBTQ	5	2
XTAPQ	5	1
BAFQ	4	2
BTAQ	4	2
BTQ	4	1
NA	0	1

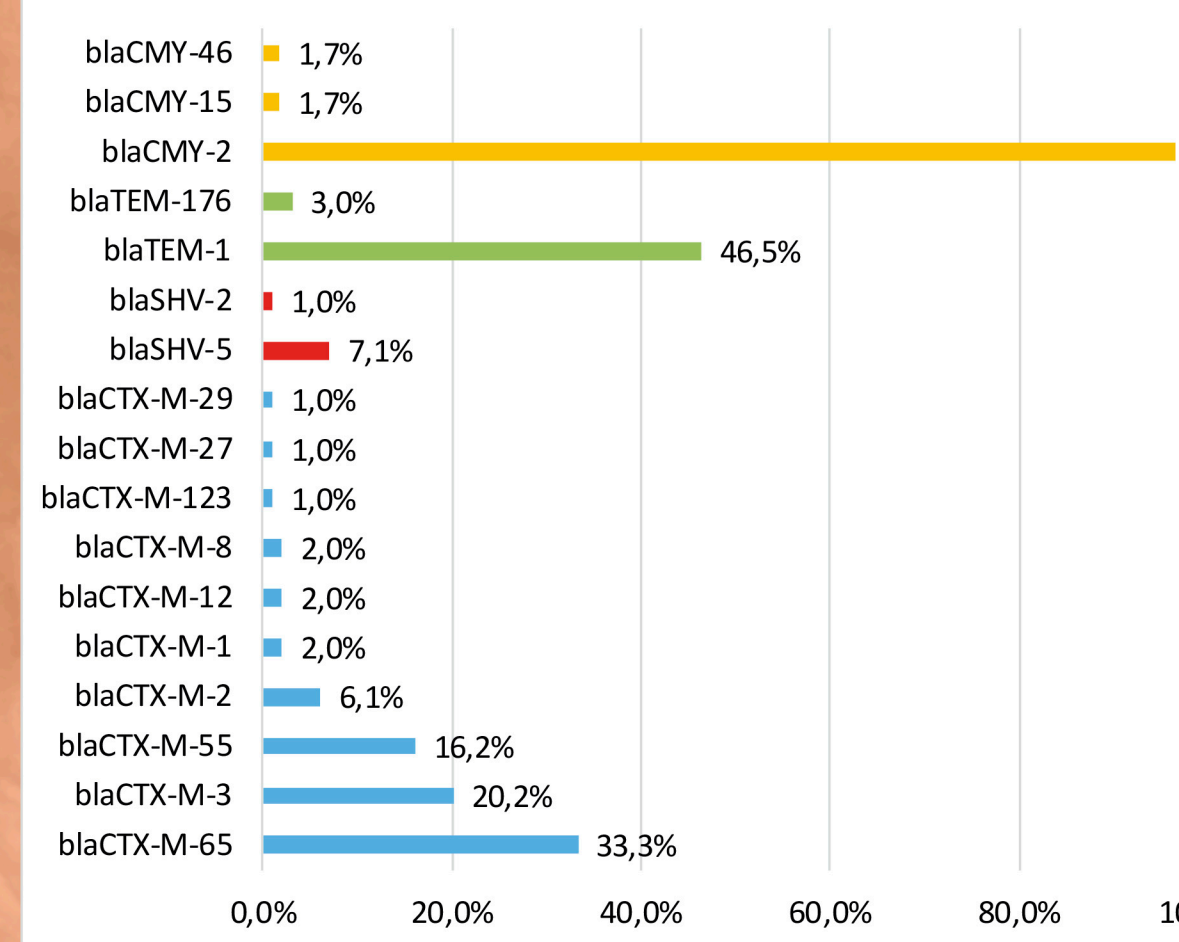
¹Folate Pathway Inhibitors (X), Tetracycline (T), Aminoglycoside (A), Phenolics (P), Nitrofurans (N), Quinolones (Q), Betalactamic (B).

Combinations of resistance genes found in *E. coli* ESBL



Antibiotic tested
Folate Pathway Inhibitors: Sulfamethoxazole + Trimethoprim
Tetracycline: Tetracycline, Doxycycline
Aminoglycoside: Streptomycin, Kanamycin, Gentamicin
Phenolics: Chloramphenicol
Nitrofurans: Nitrofurantoin
Quinolones: Nalidixic acid, Ciprofloxacin
Betalactamic: Ceftazidime, Ertapenem, Amoxicillin + Clavulanic acid

Resistance genes found in *E. coli* (AmpC and ESBL)



blaCMY genes were identified in isolates with AmpC phenotype. blaTEM, blaSHV and blaCTX were identified in isolates with ESBL phenotype.

Conclusions

This study shows the importance that these pathogens could have in the food chain in Ecuador. Reports in the region show different rates of *S. Infantis* and *C. coli* in broiler farms, which could be attributed to differences in environmental conditions and specific risk factors for flock contamination in Ecuador. High resistance rates of the 3 bacteria could be linked to the common usage of antibiotics in poultry production. Genetic types common to different farms indicate the possibility of cross contamination between farms. This evidence suggests that a stricter biosecurity should be put in place to control these microorganisms in the primary sector. Our data shows that Ecuadorian poultry production is an important hotspot of antimicrobial resistances. This novel study in Ecuador gives insights on the epidemiology of these bacteria that will be used by policy makers and researchers in the future (1,2).

References

1. Vinueza C., Wautier M., Martiny D., Cisneros M., Van Damme I., De Zutter L. Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. *Poultry Science*. 2017, 1-9.
2. Vinueza C., Cevallos M., Ron-Garrido L., Bertrand S., De Zutter L. Prevalence and Diversity of *Salmonella* Serotypes in Ecuadorian Broilers at Slaughter Age. *Plos One*. 2016, 11, 1-12.



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