



Genomic Characterization of Extended-Spectrum Cephalosporin-Resistant *Salmonella enterica* in the Colombian Poultry Chain

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Salmonella enterica serovars have been isolated from Colombian broilers and broiler meat. The aim of this study was to investigate the diversity of ESBL/pAmpC genes in extended-spectrum cephalosporin resistant *Salmonella enterica* and the phylogeny of ESBL/pAmpC-carrying *Salmonella* using Whole Genome Sequencing (WGS). A total of 260 cefotaxime resistant *Salmonella* isolates, obtained between 2008 and 2013 from broiler farms, slaughterhouses and retail, were included. Isolates were screened by PCR for ESBL/pAmpC genes. Gene and plasmid subtyping and strain Multi Locus Sequence Typing was performed *in silico* for a selection of fully sequenced isolates. Core-genome-based analyses were performed per ST encountered. *bla*_{CMY-2-like} was carried in 168 isolates, 52 carried *bla*_{CTX-M-2} group, 7 *bla*_{SHV}, 5 a combination of *bla*_{CMY-2-like}-*bla*_{SHV} and 3 a combination of *bla*_{CMY-2-like}-*bla*_{CTX-M-2} group. In 25 isolates no ESBL/pAmpC genes that were screened for were found. WGS characterization of 36 selected strains showed plasmid-encoded *bla*_{CMY-2} in 21, *bla*_{CTX-M-165} in 11 and *bla*_{SHV-12} in 7 strains. These genes were mostly carried on IncI1/ST12, IncQ1, and IncI1/ST231 plasmids, respectively. Finally, 17 strains belonged to *S. Heidelberg* ST15, 16 to *S. Paratyphi B* variant Java ST28, 1 to *S. Enteritidis* ST11, 1 to *S. Kentucky* ST152 and 1 to *S. Albany* ST292. Phylogenetic comparisons with publicly available genomes showed separate clustering of Colombian *S. Heidelberg* and *S. Paratyphi B* var. Java. In conclusion, resistance to extended-spectrum cephalosporins in *Salmonella* from Colombian poultry is mainly encoded by *bla*_{CMY-2} and *bla*_{CTX-M-165} genes. These genes are mostly associated with IncI1/ST12 and IncQ1 plasmids, respectively. Evolutionary divergence is observed between Colombian *S. Heidelberg* and *S. Paratyphi B* var. Java and those from other countries.

Keywords: Latin America, chicken, *S. Paratyphi B* d-tartrate positive, *S. Heidelberg*, *S. Java*, MLST, pMLST

INTRODUCTION

Salmonella Enteritidis and *S. Typhimurium* have been reported as the most frequent serovars causing salmonellosis in humans worldwide (Hendriksen et al., 2011). According to data collected in the European Union (EU) in 2015 and the United States (USA) in 2013, *S. Enteritidis* accounted for 46 and 15% of *Salmonella* infections and *S. Typhimurium* for 16 and 13%, respectively (Centers for Disease Control and Prevention (CDC), 2016; European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2016). Likewise, *S. Typhimurium* and *S. Enteritidis* are the most isolated serovars from human cases in Colombia. Their overall prevalence among human isolates between 2005 and 2011 was 32 and 28%, respectively (Rodríguez et al., 2017).

Among foods of animal origin, poultry products (e.g., eggs) have been primarily associated with *S. Enteritidis* infections in humans while *S. Typhimurium* infections are associated with a wider range of sources including pork, beef and poultry products (Mughini-Gras et al., 2014; Antunes et al., 2016). Nevertheless, in broilers and chicken meat the prevalence of serotypes other than *S. Enteritidis* and *S. Typhimurium* have been on the rise over the last 2 decades (van Pelt et al., 2003; Foley et al., 2011; Wagenaar et al., 2013). Baseline studies of *Salmonella* performed between 2005 and 2006 in broiler chickens in the EU showed *S. Infantis*, *S. Mbandaka*, and *S. Hadar* to be highly prevalent (European Food Safety Authority (EFSA), 2007). In the year 2006, *S. Infantis*, *S. Enteritidis* and *S. Paratyphi B* d-tartrate positive (here referred as *S. Paratyphi B* variant Java) were the most frequent serovars in broiler meat in the EU (European Food Safety Authority (EFSA), 2007). More recently, *S. Infantis*, *S. Enteritidis*, *S. Mbandaka*, and *S. Ohio* were the most prevalent serovars in broilers and broiler meat in 2015 (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2016). In North America, *S. Kentucky*, *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg*, and *S. Infantis* are reported as the most prevalent serovars in broilers and ground chicken meat in the USA (United States Department of Agriculture (USDA) - Food Safety and Inspection Service (FSIS), 2013) while in Canada, *S. Heidelberg*, *S. Kentucky* and *S. Enteritidis* are the most prevalent in broilers and broiler meat (Public Health Agency of Canada, 2016). In Colombia, baseline studies performed by the Colombian integrated program for antimicrobial resistance surveillance (Coipars), demonstrated *S. Paratyphi B* var. Java, and *S. Heidelberg* to be the most prevalent serovars in broiler farms and meat at retail. Serovar distribution at farm level was 76 and 23%, respectively (Donado-Godoy et al., 2012b), and at the retail level 45 and 19%, respectively (Donado-Godoy et al., 2014). A similar distribution of *S. Paratyphi B* var. Java and *S. Heidelberg*, has been reported in chicken meat at retail in Guatemala (35 and 16% respectively) (Jarquin et al., 2015) and broilers at slaughter in Venezuela (62 and 31% respectively) (Boscán-Duque et al., 2007).

Isolates of *S. Paratyphi B* var. Java and *S. Heidelberg* are often multi drug resistant (Denny et al., 2007; Dutil et al., 2010; Antunes et al., 2016; Liakopoulos et al., 2016) and have been associated with carriage of plasmid-mediated extended

spectrum β -lactamases (ESBLs) and plasmid associated AmpC β -Lactamases in poultry (pAmpC) (Antunes et al., 2016). For instance, *S. Paratyphi B* var. Java isolates from poultry in Europe have been found carrying the ESBL gene *bla*_{CTX-M-1} and the pAmpC gene *bla*_{CMY-2} (Doublet et al., 2014; Mevius et al., 2015; Veldman et al., 2016). In turn, *S. Heidelberg* has been associated to *bla*_{CMY-2} in North America (Andrysiak et al., 2008; Folster et al., 2012) and poultry products imported from South America into Europe (Liakopoulos et al., 2016). In South America *S. Heidelberg* has been associated with *bla*_{CTX-M-2} (Antunes et al., 2016). To date, no data of the genetic determinants of Extended Spectrum Cephalosporin (ESC) resistance in *Salmonella* from broilers and chicken meat in Colombia are available. These data are highly relevant to understand the epidemic spread of ESBL/pAmpC-producing *Salmonella* in poultry resulting in frequent occurrence in chicken meat in multiple countries.

Serotyping has traditionally been used for the epidemiological investigation of *Salmonella*, but does not provide information about the evolutionary relatedness of strains. Sequence based methodologies such as Multi Locus Sequence Typing (MLST) and Whole Genome Sequencing (WGS) have been proposed as a replacement of serotyping to identify evolutionary and epidemiological relatedness (Achtman et al., 2012; Ashton et al., 2016; Nadon et al., 2017). Additionally, information on the genetic basis of antimicrobial resistance (AMR) and plasmids harboring AMR genes can be readily obtained from WGS assemblies (Zankari et al., 2012; Carattoli et al., 2014). Altogether, the objectives of this study were to investigate the diversity of ESBL/pAmpC genes and encoding plasmids found in ESC-resistant *S. enterica* from broilers and broiler meat in Colombia and to determine the genetic relatedness with *Salmonella* strains from other countries using MLST and core-genome alignments.

MATERIALS AND METHODS

Isolates of *Salmonella enterica*

The isolates included in this study originated from different cross-sectional baseline studies conducted between 2008 and 2013 at different stages during the development of Coipars. In these studies, non-clinical samples were obtained from three different levels of poultry production in Colombia: Broiler farms (Donado-Godoy et al., 2012b), broilers at slaughter (Donado-Godoy et al., 2015b) and broiler meat at retail (Donado-Godoy et al., 2012a, 2014, 2015a). The methodology for sampling, random isolation (i.e., without antimicrobials during enrichment) of *Salmonella* and antimicrobial susceptibility testing with the BD Phoenix automated system, was previously described in detail for the studies in broiler farms, broilers at slaughter and broiler meat at retail mentioned above. Previous results of the prevalence of *Salmonella* and resistance to ESC at the different levels of poultry production, are summarized in **Table 1**.

For the present study, all available *S. enterica* isolates ($n = 673$) were considered. The 673 isolates belonged to 578 production flocks, 28 flocks from broiler farms, 140 from slaughterhouses and 410 from retail (**Table 1**). Next, ESC-resistant isolates

TABLE 1 | Prevalence and origin of *Salmonella* isolates used in this study, distribution of ESBL/pAmpC genes and selection of isolates for WGS.

	Number of samples	<i>Salmonella</i> -positive (%)	ESBL/pAmpC-producing <i>Salmonella</i> (%) ^a	<i>bla</i> _{CMY-2-like}	<i>bla</i> _{CTX-M-2} group	<i>bla</i> _{SHV} family	<i>bla</i> _{CMY-2-like} - <i>bla</i> _{CTX-M-2} group	<i>bla</i> _{CMY-2-like} - <i>bla</i> _{SHV} family
Farms	74	28 (38)	17 (61)	16/2 ^b	–	–	–	1/1 ^b
2008	65	23	17	16	–	–	–	1
2009	9	5	–	–	–	–	–	–
Slaughter	644	140 (22)	82 (59)	54/4 ^b	25/2 ^b	–	2/2 ^b	1/1 ^b
2008	40	5	3	3	–	–	–	–
2012	251	76	39	31	8	–	–	–
2013	353	59	40	20	17	–	2	1
Retail	1554	410 (26)	136 (33)	98/7 ^b	27/6 ^b	7/7 ^b	1/1 ^b	3/3 ^b
2009	179	38	11	11	–	–	–	–
2010	387	156	35	29	1	4	–	1
2011	823	170	76	49	22	2	1	2
2012	165	46	14	9	4	1	–	–
Total	2272	578 (25)	235 (41)	168/13 ^b	52/8 ^b	7/7 ^b	3/3 ^b	5/5 ^b

^aBased on PCR screening of ESBL/pAmpC genes.

^bESBL/pAmpC-positive samples/Selected for whole genome sequencing.

of *Salmonella* were selected based on suspected phenotypic resistance to cefotaxime, as previously measured with the BD Phoenix (Donado-Godoy et al., 2012b) and interpreted using the CLSI 2014 clinical breakpoints for Enterobacteriaceae (MIC $\geq 4 \mu\text{g/ml}$) (Clinical and Laboratory Standards Institute (CLSI), 2014). For all flocks with multiple ESC-resistant isolates, each first isolate was selected to make sure we included epidemiologically unrelated isolates only.

As a result, a total of 260 isolates were selected for ESC-resistance characterization. The selected isolates from farms originated from drag swabs of fecal material from the floor of broiler houses and fresh feces directly from the chicken. These isolates originated from 19 broiler farms ($n = 19$) (Donado-Godoy et al., 2012b). Isolates at slaughter, originated from cecal content and carcass rinse from 32 slaughterhouses ($n = 84$) (Donado-Godoy et al., 2015b). At retail, isolates originated from carcass rinse from 143 retail suppliers (Donado-Godoy et al., 2012a, 2014) and chicken thighs meat from 8 retail suppliers ($n = 157$) (Donado-Godoy et al., 2015a). The isolates originated from 18 out of 32 departments (i.e. provinces) of Colombia. Together, these 18 departments are responsible for more than 90% of the chicken population in the country. A map of Colombia with the location of these departments is available in **Supplementary Figure 1**.

Information related to the origin of the samples and the prevalence of *Salmonella* is shown in **Table 1**.

PCR Screening of ESBL/pAmpC Genes

For the present investigation, the 260 isolates selected as mentioned above, were screened by PCR for the presence of ESBL/pAmpC genes families using previously described primers for *bla*_{TEM} and *bla*_{SHV} families, *bla*_{CMY-2-like} and *bla*_{CTX-M} family (Dierikx et al., 2010); *bla*_{CTX-M-1} group (Carattoli et al., 2008); *bla*_{CTX-M-2} group (Jiang et al., 2006); *bla*_{CTX-M-8} group (Hopkins et al., 2006); *bla*_{CTX-M-9} group (Paauw et al., 2006);

*bla*_{OXA-1-like}, *bla*_{OXA-2-like} and *bla*_{OXA-10-like} (Voets et al., 2011).

Selection of Strains and WGS

Based on the diversity of gene families found after PCR screening, a representative selection of isolates was made and further subjected to WGS. Due to its high prevalence, a random selection was made using the Random function in Microsoft[®] Excel to assign random numbers to isolates positive for *bla*_{CMY-2-like} and *bla*_{CTX-M-2} group. The size of selection was fixed to the square root of the number of resulting positive strains for these genes. This resulted in 13 *bla*_{CMY-2-like}- and 8 *bla*_{CTX-M-2} group-carrying isolates. In addition and due to their low prevalence, all positive isolates for *bla*_{SHV} family and combinations of *bla*_{CMY-2-like}-*bla*_{SHV} family and *bla*_{CMY-2-like}-*bla*_{CTX-M-2} group were included. An overview of the selection of isolates is shown in **Table 1**.

Isolation of genomic DNA from selected isolates was performed using the UltraClean[®] Microbial DNA Isolation Kit (Mo Bio-Qiagen, USA). WGS was performed on Illumina MiSeq and NextSeq platforms (Illumina, USA) using 2×250 -bp reads and 2×150 -bp reads, respectively. Genomes were assembled with SPAdes v3.10.1 (Bankevich et al., 2012).

In silico Characterization of ESBL/pAmpC Gene Variants

Subtyping of ESBL/pAmpC gene variants was performed using ResFinder 2.1 (Zankari et al., 2012). Investigation of resistance genes with an identity percentage $< 100\%$ was done using BLAST 2.6.0+ (Camacho et al., 2009).

In silico Characterization of ESBL/pAmpC-Carrying Plasmids

Plasmid content of selected strains was investigated using PlasmidFinder 1.3 and pMLST 1.4 (Carattoli et al., 2014).

Identification of plasmids associated to the ESBL/pAmpC genes was based on co-localization on the same contig as resulted from ResFinder and PlasmidFinder analysis. Since this was not possible for all genomes, transformation of plasmids harboring each of the ESBL/pAmpC variants identified with ResFinder was performed together with selective culturing as described before (Castellanos et al., 2017). This was done to obtain the plasmid types identified with PlasmidFinder in transformed *Escherichia coli* DH10B harboring the different ESBL/pAmpC variants identified. Afterwards, the sequences of the transformed plasmids were used as reference to map against the genomes of the selected strains. To this purpose, transformants were sequenced with Illumina MiSeq and NextSeq sequencing as described above. Chromosomal contigs in transformants were detected and removed by mapping against the *E. coli* DH10B genome sequence (GenBank accession number: CP000948.1) using BLAST. The remaining contigs were considered to be part of the ESBL/pAmpC carrying plasmid and were used as a reference. Next, the contigs of the initially sequenced selected strains were aligned to the obtained reference plasmid sequences using MUSCLE (Edgar, 2004). Alignments were made on selected strains according to the ESBL/pAmpC gene variants they harbored. Resulting aligned contigs were selected and considered as newly inferred ESBL/pAmpC-carrying plasmids.

In silico MLST and Serotype Prediction

To determine the population structure of the selected ESC-resistant *Salmonella*, 7-gene MLST at the strain level was performed *in silico* with MLST 1.8 (Larsen et al., 2012). Serotype was predicted using the *Salmonella In Silico* Typing Resource (SISTR) (Yoshida et al., 2016). Whole genome phylogenetic analyses were performed for *S. Heidelberg* ST15 and *S. Paratyphi* B var. Java ST28. Given the limited number of isolates ($n = 1$), this was not performed for *S. Enteritidis* ST11, *S. Kentucky* ST152 or *S. Albany* ST292.

Collection of Publicly Available Genomes for Phylogenetic Comparisons

Genome sequences of *S. Paratyphi* B var. Java ST28, were downloaded from Enterobase¹ (last accessed: 12-Sept-2017) for comparison. Likewise, sequences of *S. Heidelberg* ST15 were obtained from Enterobase¹ (last accessed: 11-Jan-2017). Only genomes with data available for their year of isolation, country of origin and source were considered for *S. Heidelberg* ST15. For both *S. Paratyphi* B var. Java ST28 and *S. Heidelberg* ST15, the genomes were collected disregarding their susceptibility to 3rd generation cephalosporin's or to other antimicrobials. Additionally, the quality of genomes obtained in this study and the downloaded genomes was assessed with CheckM (Parks et al., 2015). Only genomes with >98% completeness score, when compared against the set of genomic markers for *S. enterica*, were included. MLST designation was amended using a custom BLAST-based tool².

¹<https://enterobase.warwick.ac.uk/>

²<https://github.com/tseemann/mlst>

Core Genome Phylogenetic Analysis

Whole genome analysis was performed by a core-genome alignment using Parsnp v1.2 (Treangen et al., 2014). Recombination regions in the core genome alignment were detected and filtered using Gubbins (Croucher et al., 2015). Phylogenetic maximum likelihood (mid-point rooted) trees were constructed with the recombination-filtered core genomes alignments using FastTree2³ (Price et al., 2010) and visualized with FigTree⁴.

Data Availability

The obtained genome sequences of the *Salmonella* strains selected for WGS (Table 2) and those of the transformed *E. coli* DH10B strain with the ESBL/pAmpC-carrying plasmids used as reference have been deposited in the short-read archive of the ENA under Project Number: PRJEB23610.

RESULTS

PCR Screening of ESBL/pAmpC Genes

After PCR screening of the 260 isolates, 235 isolates were positive for the genes screened for. 168 were positive for *bla*_{CMY-2-like}, 52 for *bla*_{CTX-M-2} group, 7 for *bla*_{SHV} family, 5 harbored a combination of *bla*_{CMY-2-like}-*bla*_{SHV} family and 3 a combination of *bla*_{CMY-2-like}-*bla*_{CTX-M-2} group. In 48 isolates, *bla*_{TEM} was co-located with *bla*_{CMY-2-like}, *bla*_{CTX-M-2} group or *bla*_{SHV}. In 25 isolates no ESBL/pAmpC genes that were screened for were encountered.

The distribution of positive samples for *Salmonella* according to their source, year and ESBL/pAmpC genes is shown in Table 1.

Selection of Strains for WGS

After PCR characterization, a random selection of *bla*_{CMY-2-like}-positive isolates ($n = 13$) and *bla*_{CTX-M-2} group ($n = 8$) together with all positive isolates for *bla*_{SHV} family ($n = 7$), *bla*_{CMY-2-like}-*bla*_{SHV} family ($n = 5$) and *bla*_{CMY-2-like}-*bla*_{CTX-M-2} group ($n = 3$) were included for WGS. In total, 36 isolates were selected and subjected to WGS. The list of selected isolates and the results of the characterization of ESBL/pAmpC genes, plasmid types, serotypes, and strain MLST based on WGS is shown in Table 2.

Characterization of ESBL/pAmpC Gene Variants

After WGS characterization of resistance genes, 13 strains harbored *bla*_{CMY-2}, 8 *bla*_{CTX-M-165}, 6 *bla*_{SHV-12} and 1 *bla*_{SHV-129}. Additionally, three harbored the combination of *bla*_{CMY-2}-*bla*_{CTX-M-165}, four *bla*_{CMY-2}-*bla*_{SHV-129} and one *bla*_{CMY-2}-*bla*_{SHV-12} (Table 2). All accompanying *bla*_{TEM} variants were identified as *bla*_{TEM-1A} or *bla*_{TEM-1B}.

In silico Characterization of ESBL/pAmpC-Carrying Plasmids

Co-localization of ESBL/pAmpC and plasmid replicon genes in the same contig was observed for 17 out of 36 strains selected for

³<http://meta.microbesonline.org/fasttree/>

⁴<http://tree.bio.ed.ac.uk/software/figtree/>

TABLE 2 | Origin of the *Salmonella* isolates selected for WGS and results of the characterization of their ESBL/pAmpC genes, plasmids, and strains.

Source	Year	Strain	Location (Department)	Accession number ENA	β -lactam resistance genes ^a	ESBL/pAmpC-harboring plasmid ^c	<i>S. enterica</i> Serovar	<i>S. enterica</i> MLST
Farm	2008	SSXXXV.4.C1	Santander	ERS2017899	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
		SSIII.4.C2	Santander	ERS2017900	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{SHV-129}</u> ^b <u><i>bla</i>_{TEM-1A}</u>	<u>Incl1/ST12</u>	S. Java	ST28
Slaughter	2012	SXXI.1.C5	Cundinamarca	ERS2017901	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
		FBOG8	Bogotá	ERS2017931	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
		FSAN161	Santander	ERS2017935	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
		FSAN236	Santander	ERS2017936	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
	2013	FBOG7	Bogotá	ERS2017938	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1^d</u>	S. Java	ST28
		FSUC414	Sucre	ERS2017932	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{CTX-M-165}</u> ^e <u><i>bla</i>_{TEM-1B}</u>	<u>Incl1/ST12-IncQ1^e</u>	S. Heidelberg	ST15
		FCAR509	Bolívar	ERS2017933	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Heidelberg	ST15
		FANT596	Antioquia	ERS2017934	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{SHV-129}</u> ^b <u><i>bla</i>_{TEM-1B}</u>	<u>Incl1/ST12</u>	S. Java	ST28
		FVAL369	Valle del Cauca	ERS2017937	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Heidelberg	ST15
		FPAS506	Nariño	ERS2017939	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{CTX-M-165}</u>	<u>Incl1/ST12-Non-typeable^e</u>	S. Heidelberg	ST15
		Retail	2010	UGBOG4	Bogotá	ERS2017904	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{SHV-12}</u>	<u>Incl1/ST12-Non-typeable^e</u>
UGBOG316	Bogotá			ERS2017908	<u><i>bla</i>_{SHV-12}</u>	Non-typeable	S. Heidelberg	ST15
UGBOG327	Bogotá			ERS2017909	<u><i>bla</i>_{SHV-12}</u>	<u>Incl1/ST231</u>	S. Java	ST28
UGBOG339	Bogotá			ERS2017910	<u><i>bla</i>_{SHV-12}</u>	<u>Incl1/ST231</u>	S. Java	ST28
2011	UGBOG340		Bogotá	ERS2017911	<u><i>bla</i>_{SHV-12}</u>	<u>Incl1/ST231</u>	S. Java	ST28
	UGBAR394		Atlántico	ERS2017912	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{CTX-M-165}</u> ^e <u><i>bla</i>_{TEM-1B}</u>	<u>Incl1/ST12-IncQ1^e</u>	S. Heidelberg	ST15
	UGBAR434		Atlántico	ERS2017913	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Albany	ST292
	UGCAR507		Bolívar	ERS2017914	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
	UGVAL515		Valle del Cauca	ERS2017915	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{SHV-129}</u>	<u>Incl1^d</u>	S. Heidelberg	ST15
	UGBUC832		Santander	ERS2017916	<u><i>bla</i>_{SHV-129}</u>	-	S. Heidelberg	ST15
	UGCUC851		Norte de Santander	ERS2017917	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
	UGCUC867		Norte de Santander	ERS2017918	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
	UGARA888		Arauca	ERS2017919	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Enteritidis	ST11
	UGIBA933		Tolima	ERS2017920	<u><i>bla</i>_{SHV-12}</u>	<u>Incl1/ST231</u>	S. Java	ST28
	UGPER971		Risaralda	ERS2017921	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
	UGBOG1024		Bogotá	ERS2017922	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Java	ST28
	UGPAS1097		Nariño	ERS2017923	<u><i>bla</i>_{CMY-2}</u>	<u>Incl1/ST12</u>	S. Kentucky	ST152
	UGBUC1112		Santander	ERS2017924	<u><i>bla</i>_{CTX-M-165}</u>	Non-typeable	S. Heidelberg	ST15
	UGBUC1123		Santander	ERS2017925	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
	UGBAR1160		Atlántico	ERS2017926	<u><i>bla</i>_{CTX-M-165}</u>	<u>IncQ1</u>	S. Heidelberg	ST15
UGBAR1170	Atlántico	ERS2017927	<u><i>bla</i>_{CMY-2}</u> - <u><i>bla</i>_{SHV-129}</u> ^b <u><i>bla</i>_{TEM-1B}</u>	<u>Incl1/ST12</u>	S. Java	ST28		
2012	UGBAR1187	Atlántico	ERS2017928	<u><i>bla</i>_{CTX-M-165}</u> - <u><i>bla</i>_{TEM-1B}</u>	<u>IncQ1</u>	S. Heidelberg	ST15	
	UGBOG1279	Bogotá	ERS2017929	<u><i>bla</i>_{SHV-12}</u>	Non-typeable	S. Heidelberg	ST15	
	UGBOG1280	Bogotá	ERS2017930	<u><i>bla</i>_{CMY-2}</u>	Non-typeable	S. Java	ST28	

^aThe genes present in the contigs selected for plasmid characterization have been underlined.

^b*bla*_{SHV-129} was non-transferable after electroporation experiments.

^cThe plasmids carrying the β -lactam resistance genes have been underlined.

^dThese plasmids missed one allele from the pMLST scheme.

^eCharacterization of the two ESBL/pAmpC genes was performed in separate plasmid contigs.

WGS. For the remaining strains, co-localization was determined by analyzing selected contigs harboring ESBL/pAmpC and plasmid replicon genes using MUSCLE. Genes conferring resistance to other antimicrobials only co-localized with *bla*_{CTX-M-165}-harboring contigs, not with other ESBL/pAmpC genes (**Supplementary Table 1**). In detail, one transformant was obtained carrying either a *bla*_{CMY-2}, *bla*_{CTX-M-165}- or *bla*_{SHV-12}-harboring plasmid. The plasmids isolated from strains UGBOG4 (*bla*_{CMY-2}), UGBAR1160 (*bla*_{CTX-M-165}) and UGBOG327 (*bla*_{SHV-12}) (**Supplementary Table 1**) were used as a reference to map against the genomes of all selected strains. The *bla*_{SHV-129} gene present in five selected strains was not transferable by transformation from any of the strains, suggesting chromosomal localization of this gene.

After characterizing the plasmid contigs of the selected strains, 18 of 21 *bla*_{CMY-2}-carrying plasmids were found to belong to IncI1/ST12, 1 was non-typeable based on the PCR Based Replicon Typing (PBRT) scheme used in PlasmidFinder (Carattoli et al., 2014). Two were designated IncI1, but the plasmid from strain UGVAL515 lacked the *pill* allele and the plasmid from FBOG7 lacked the *sogS* allele. Nonetheless, these two plasmids remained single-allele variants of IncI1/ST12. For *bla*_{CTX-M-165}, 9 of 11 plasmids harbored the IncQ1 plasmid-replicon and 2 remained non-typeable. Four of seven *bla*_{SHV-12} plasmids belonged to IncI1/ST231 and three remained non-typeable (**Table 2** and **Supplementary Table 1**).

Strain MLST, Serotype Characterization, and Core Genome Phylogeny

After using 7-gene MLST and the *Salmonella In Silico* Typing Resource (SISTR), 17 strains belonged to *S. Heidelberg* ST15, 16 to *S. Paratyphi B* var. Java ST28, 1 to *S. Enteritidis* ST11, 1 to *S. Kentucky* ST152 and 1 to *S. Albany* ST292 (**Table 2**). Further, whole genome analysis was performed for ST28 and ST15 isolates. For the phylogenetic analysis, additional genomes for ST28 ($n = 60$) and ST15 ($n = 1221$) were selected from Enterobase.warwick.ac.uk disregarding their characteristics of susceptibility to 3rd generation cephalosporins and used to construct the phylogenetic maximum likelihood trees. All Colombian genomes belonging to ST28 and ST15 formed a single cluster in the phylogenetic analysis. Phylogenetic trees for ST28 and ST15 with data regarding the source, year and country of the strains, are shown in **Figures 1** and **2** respectively. No clustering related to the presence or absence of an ESBL/pAmpC gene is observed in **Figure 1**, suggesting the observed clustering is related to the geographical origin of *S. Paratyphi B* var. Java ST28 strains and not to the presence of an AMR gene. Likewise, in **Figure 2**, a cluster of *S. Heidelberg* ST15 strains originating from Colombian poultry is observed. Furthermore, the Colombian strains from ST28 and ST15 were disseminated in multiple departments within the country. A map of Colombia with the location of origin of ST28 and ST15 isolates selected for WGS is available in **Supplementary Figure 2**. An additional table with the metadata of strains selected for the construction of the ST28 and ST15 phylogenies is available as **Supplementary Table 2**.

DISCUSSION

In summary, *bla*_{CMY-2}, *bla*_{CTX-M-165}, *bla*_{SHV-12}, and *bla*_{SHV-129} are described as the most prevalent ESBL/pAmpC genes conferring resistance to ESC in *S. enterica* isolated from the Colombian poultry chain between 2008 and 2013. According to the objectives of the present study, the collection of isolates served to reflect maximum diversity of ESBL/pAmpC genes in different years, departments and levels of the poultry production chain in Colombia.

The finding of *bla*_{CMY-2} as the main cause of ESC resistance is comparable to a previous report of ESBL/pAmpC-producing *E. coli* in Colombian poultry. In that study, it was encountered a prominent association of this gene with IncI1/ST12 plasmids (Castellanos et al., 2017). Those results suggested occurrence of horizontal gene transfer of this plasmid lineage between heterogeneous *E. coli* STs. The results from the present study, suggest that transfer of *bla*_{CMY-2}-IncI1/ST12 plasmids between *E. coli* and *Salmonella* is also likely to occur, and could be considered a driver of the frequent occurrence of this resistance gene along the poultry chain. This particular gene-plasmid association has been described in *E. coli* from Brazil (da Silva et al., 2017), *Salmonella* from the USA (Folster et al., 2010) and *E. coli* and *Salmonella* from Europe (Accogli et al., 2013; Smith et al., 2015), suggesting an epidemiological link between the presence of *bla*_{CMY-2}-IncI1/ST12 and poultry from different countries. Nevertheless, WGS of these plasmids is necessary to assess the level of genetic relatedness among them and estimate the potential transmission of these plasmids between *E. coli* and *Salmonella*.

After PCR screening, *bla*_{CTX-M-2} group was found to be the most prevalent ESBL gene among Colombian isolates. After subtyping a selection of these isolates with WGS, these genes were found to be *bla*_{CTX-M-165}. To date, this variant has been solely reported in an isolate of *Klebsiella pneumoniae* from a urine sample in Chile and reported in 2016 (Accession number: KP727572) without further epidemiological records. Noticeably, all *bla*_{CTX-M-165}-positive strains, alone or in combination with other *bla* genes, were identified in *S. Heidelberg* ST15. In our collection of isolates comprising the years 2008 to 2013, *bla*_{CTX-M-165} is only detected from the year 2010 onwards (**Table 1**). Not taking into account the potential bias that could occur by having isolates comprising a period of time no longer than 5 years, this finding may suggest a recent introduction of this gene in Colombian poultry, and until 2013, is limited to *S. Heidelberg* ST15. However, analysis of recently collected isolates of *S. enterica* and other Enterobacteriaceae is necessary to confirm this hypothesis.

After electroporation experiments, *bla*_{SHV-129} remained non-transferable and no plasmid markers were identified in its harboring contigs, which ranged in size between 2100 and 8594 bp. Therefore, it is likely that this gene is chromosomally located. Furthermore, the gene was found in two different serovars, *S. Paratyphi B* var. Java ST28, and *S. Heidelberg* ST15. It can be hypothesized that its transfer could be associated to an integrative or transposable element. Initial screening of transposases and Insertion Sequences (IS) using BLAST

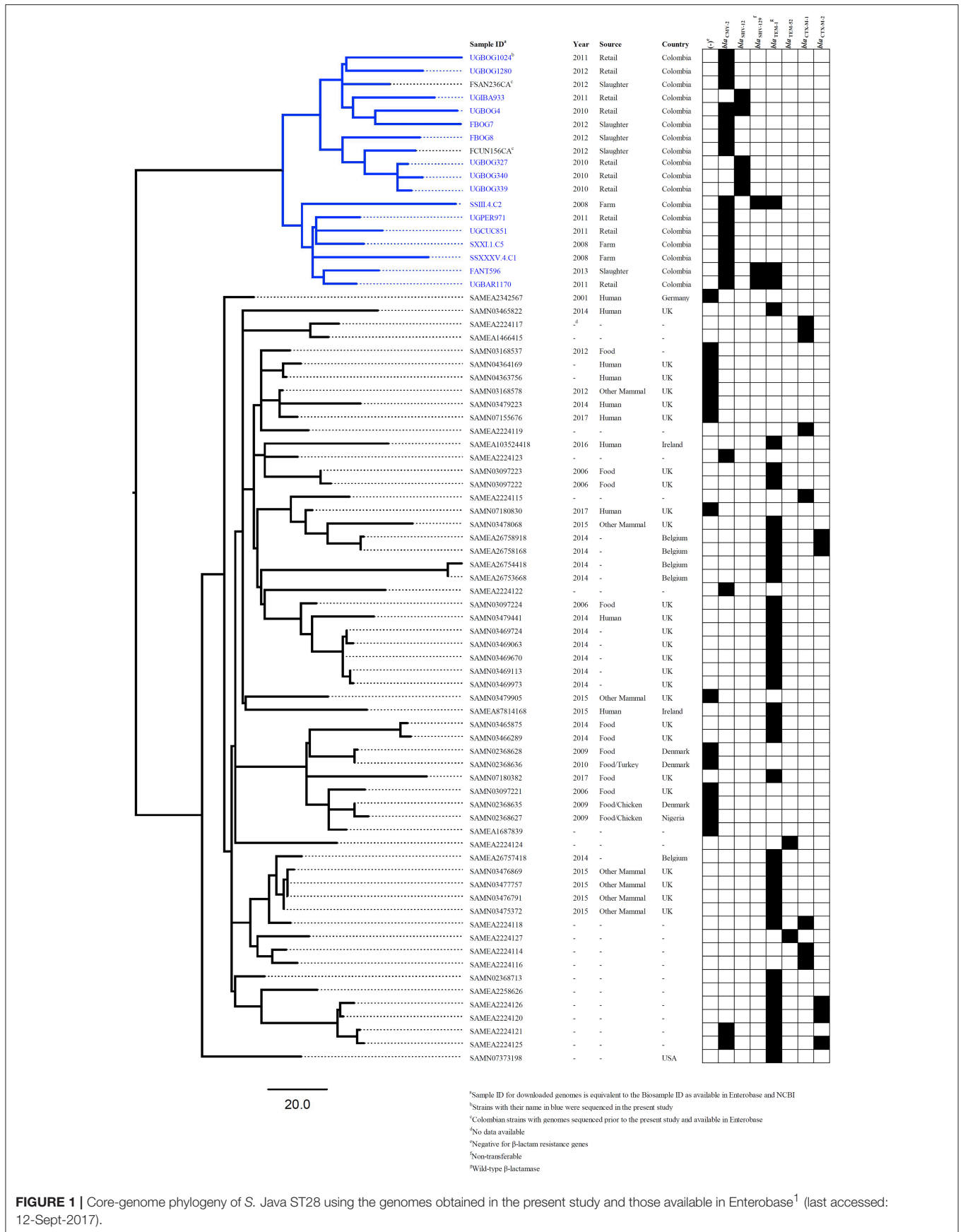
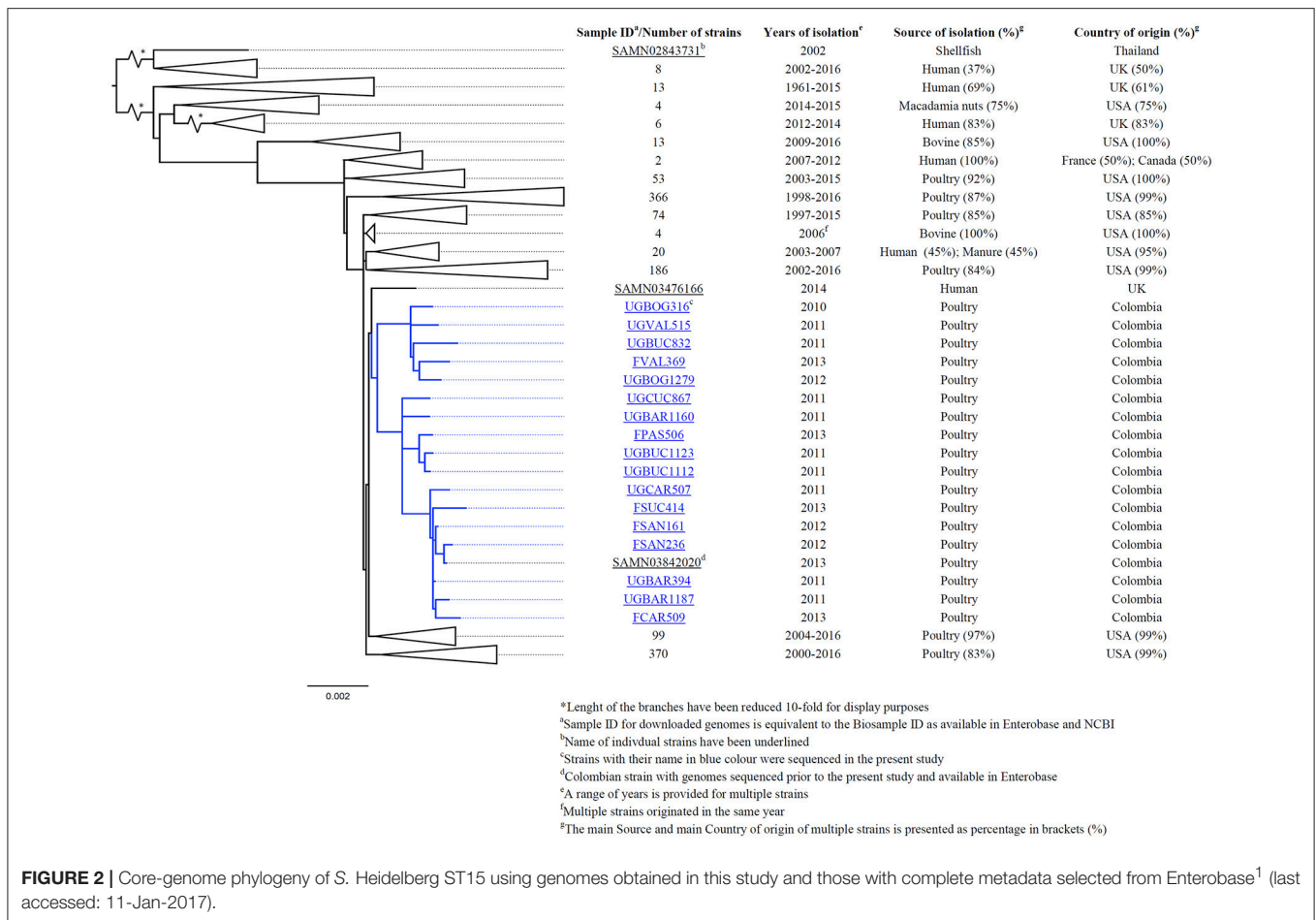


FIGURE 1 | Core-genome phylogeny of *S. Java* ST28 using the genomes obtained in the present study and those available in Enterobase¹ (last accessed: 12-Sept-2017).



(Siguier, 2006) on the contigs harboring these genes detected several IS families flanking the sequences inside the contigs. Nevertheless, given the restricted size of the contigs no definite association with a unique IS element (e.g., AMR-associated IS) was possible. In such a case, complementing the short-read WGS data with additional data obtained through long-read sequencing is necessary to confirm the chromosomal location of this gene and its association to a particular mobile genetic element. This approach could also be used for further characterization of ESBL/AmpC-plasmids that were non-typeable according to the PBRT scheme used in PlasmidFinder, which could have also been affected by the limitations of genome and plasmid assembly of short-reads.

As mentioned previously, ESC and non-ESC *S. Paratyphi* B var. Java and *S. Heidelberg* were reported as the most prevalent serovars in the Colombian poultry chain (Donado-Godoy et al., 2012b, 2014). In the present study, investigation of resistance to cefotaxime showed a total of 235 (41%) resistant isolates from which, 17 (61%) originated from farms, 82 (59%) from slaughterhouses and 136 (33%) from retail (Table 1). Noteworthy, the prevalence of resistance diminishes from one level of production to the other. From previous studies, *S. Paratyphi* B var. Java and *S. Heidelberg* were the most prevalent

serovars encountered at the farm level (Donado-Godoy et al., 2012b) and a larger diversity of serovars was found at retail, with more than 10 different serovars isolated repeatedly (Donado-Godoy et al., 2014). As observed in the present study, strains belonging to *S. Paratyphi* B var. Java and *S. Heidelberg* had a higher prevalence of ESC-resistance in comparison to the other serovars. These results indicate that the higher prevalence of *S. Paratyphi* B var. Java and *S. Heidelberg*, accounted in large part for the higher prevalence of ESC-resistance at the farm level and the presence of different serovars resulted in the reduction of resistance along the production chain, which is reflected in the lower prevalence of ESC-resistance at retail.

As anticipated, the analysis of *Salmonella* strains using MLST in addition to the resolution provided by WGS data has proven to be very useful in showing clustering of Colombian strains belonging to *S. Paratyphi* B var. Java ST28. According to the phylogenetic analysis including ESBL/pAmpC-positive and-negative strains (Figure 1), the clustering seems to occur independently of ESC-susceptibility and may be related to the geographical origin of the strains. Whether the cluster of Colombian *S. Paratyphi* B var. Java ST28 represents a particular separate lineage circulating in the country, or is present elsewhere

in Latin America is a question that requires further investigation. At the time of publication of the present study no genomes of ST28 from other Latin American countries were publicly available and the comparisons within the region were limited to the strains we sequenced and analyzed.

In conclusion, resistance to ESC in *S. enterica* from Colombian poultry is mainly caused by *bla*_{CMY-2} and *bla*_{CTX-M-165} genes. These genes are mostly associated with IncI1/ST12 and IncQ1 plasmids, respectively. The resolution provided by WGS was appropriate to assess the evolutionary divergence of strains from Colombian poultry belonging to *S. Paratyphi B* var. Java ST28. Dissemination of ESBL/pAmpC genes in *Salmonella* is mainly due to the carriage of plasmids encoding these genes in strains belonging to *S. Paratyphi B* var. Java ST28 and *S. Heidelberg* ST15.

AUTHOR CONTRIBUTIONS

JW, PD-G, JH, AZ, DM, LG-vB, and LC contributed to the design of the study. LG-vB and AZ performed the formal analysis. JW Contributed funding acquisition. LG-vB, PD-G, ML, VC, AA, JB, AZ, and LC conducted material and data collection. JW, PD-G, and AZ provided biological, laboratory and computational

resources. JH, JW, DM, and AZ supervised the study. LC wrote the original draft. JH, JW, DM, LG-vB, and AZ critically reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2018.02431/full#supplementary-material>

REFERENCES

- Accogli, M., Fortini, D., Giufrè, M., Graziani, C., Dolejska, M., Carattoli, A., et al. (2013). IncI1 plasmids associated with the spread of CMY-2, CTX-M-1 and SHV-12 in *Escherichia coli* of animal and human origin. *Clin. Microbiol. Infect.* 19:E238–E240. doi: 10.1111/1469-0691.12128
- Achtman, M., Wain, J., Weill, F. X., Nair, S., Zhou, Z., Sangal, V., et al. (2012). Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog.* 8:e1002776. doi: 10.1371/journal.ppat.1002776
- Andrysiak, A. K., Olson, A. B., Tracz, D. M., Dore, K., Irwin, R., Ng, L. K., et al. (2008). Genetic characterization of clinical and agri-food isolates of multi drug resistant *Salmonella enterica* serovar Heidelberg from Canada. *BMC Microbiol.* 8:89. doi: 10.1186/1471-2180-8-89
- Antunes, P., Mourão, J., Campos, J., and Peixe, L. (2016). Salmonellosis: the role of poultry meat. *Clin. Microbiol. Infect.* 22, 110–121. doi: 10.1016/j.cmi.2015.12.004
- Ashton, P. M., Nair, S., Peters, T. M., Bale, J. A., Powell, D. G., Painset, A., et al. (2016). Identification of *Salmonella* for public health surveillance using whole genome sequencing. *PeerJ* 4:e1752. doi: 10.7717/peerj.1752
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021
- Boscán-Duque, L. A., Arzálluz-Fisher, A. M., Ugarte, C., Sánchez, D., Wittum, T. E., and Hoet, A. E. (2007). Reduced susceptibility to quinolones among *Salmonella* serotypes isolated from poultry at slaughter in Venezuela. *J. Food Prot.* 70, 2030–2035. doi: 10.4315/0362-028X-70.9.2030
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., et al. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. doi: 10.1186/1471-2105-10-421
- Carattoli, A., García-Fernández, A., Varesi, P., Fortini, D., Gerardi, S., Penni, A., et al. (2008). Molecular epidemiology of *Escherichia coli* producing extended-spectrum β -lactamases isolated in Rome, Italy. *J. Clin. Microbiol.* 46, 103–108. doi: 10.1128/JCM.01542-07
- Carattoli, A., Zankari, E., García-Fernández, A., Larsen, M. V., Lund, O., Villa, L., et al. (2014). *In Silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* 58, 3895–3903. doi: 10.1128/AAC.02412-14
- Castellanos, L. R., Donado-Godoy, P., León, M., Clavijo, V., Arevalo, A., Bernal, J. F., et al. (2017). High heterogeneity of *Escherichia coli* sequence types harbouring ESBL/AmpC genes on IncI1 plasmids in the Colombian poultry chain. *PLoS ONE* 12:e0170777. doi: 10.1371/journal.pone.0170777
- Centers for Disease Control and Prevention (CDC). (2016). *Centers for Disease Control and Prevention National Salmonella Surveillance Annual Report, 2013. 1–89*. Available online at: <https://www.cdc.gov/nationalsurveillance/pdfs/salmonella-annual-report-2013-508c.pdf>
- Clinical and Laboratory Standards Institute (CLSI). (2014). *Clinical and Laboratory Standards Institute M100-S24. Performance Standards for Antimicrobial Susceptibility Testing. 24th Informational Supplement*. Wayne, PA: Clinical and Laboratory Standards Institute.
- Croucher, N. J., Page, A. J., Connor, T. R., Delaney, A. J., Keane, J. A., Bentley, S. D., et al. (2015). Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res.* 43:e15. doi: 10.1093/nar/gku1196
- da Silva, K. C., Cunha, M. P. V., Cerdeira, L., de Oliveira, M. G. X., de Oliveira, M. C. V., Gomes, C. R., et al. (2017). High-virulence CMY-2- and CTX-M-2-producing avian pathogenic *Escherichia coli* strains isolated from commercial turkeys. *Diagn. Microbiol. Infect. Dis.* 87, 64–67. doi: 10.1016/j.diagmicrobio.2016.10.001
- Denny, J., Threlfall, J., Takkinen, J., Lofdahl, S., Westrell, T., Varela, C., et al. (2007). Multinational *Salmonella* Paratyphi B variant Java (*Salmonella* Java) outbreak, August - December 2007. *Euro Surveill.* 12:E071220.2. doi: 10.2807/esw.12.51.03332-en
- Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., and Mevius, D. (2010). Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Vet. Microbiol.* 145, 273–278. doi: 10.1016/j.vetmic.2010.03.019
- Donado-Godoy, P., Byrne, B. A., León, M., Castellanos, R., Vanegas, C., Coral, A., et al. (2015a). Prevalence, resistance patterns, and risk factors for antimicrobial resistance in bacteria from retail chicken meat in Colombia. *J. Food Prot.* 78, 751–759. doi: 10.4315/0362-028X.JFP-14-349
- Donado-Godoy, P., Castellanos, R., León, M., Arevalo, A., Clavijo, V., Bernal, J., et al. (2015b). The establishment of the Colombian Integrated Program for

- Antimicrobial Resistance Surveillance (COIPARS): a pilot project on poultry farms, slaughterhouses and retail market. *Zoonoses Public Health* 62, 58–69. doi: 10.1111/zph.12192
- Donado-Godoy, P., Clavijo, V., León, M., Arevalo, A., Castellanos, R., Bernal, J., et al. (2014). Counts, serovars, and antimicrobial resistance phenotypes of *Salmonella* on raw chicken meat at retail in Colombia. *J. Food Prot.* 77, 227–235. doi: 10.4315/0362-028X.JFP-13-276
- Donado-Godoy, P., Clavijo, V., León, M., Tafur, M. A., Gonzales, S., Hume, M., et al. (2012a). Prevalence of *Salmonella* on retail broiler chicken meat carcasses in Colombia. *J. Food Prot.* 75, 1134–1138. doi: 10.4315/0362-028X.JFP-11-513
- Donado-Godoy, P., Gardner, I., Byrne, B. A., León, M., Perez-Gutierrez, E., Ovalle, M. V., et al. (2012b). Prevalence, risk factors, and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. *J. Food Prot.* 75, 874–883. doi: 10.4315/0362-028X.JFP-11-458
- Doublet, B., Praud, K., Nguyen-Ho-Bao, T., Argudin, M. A., Bertrand, S., Butaye, P., et al. (2014). Extended-spectrum β -lactamase and ampc β -lactamase-producing D-tartrate-positive *Salmonella enterica* serovar Paratyphi B from broilers and human patients in Belgium, 2008–10. *J. Antimicrob. Chemother.* 69, 1257–1264. doi: 10.1093/jac/dkt504
- Dutil, L., Irwin, R., Finley, R., Ng, L. K., Avery, B., Boerlin, P., et al. (2010). Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerging Infect. Dis.* 16, 48–54. doi: 10.3201/eid1601.090729
- Edgar, R. C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113. doi: 10.1186/1471-2105-5-113
- European Food Safety Authority (EFSA) (2007). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. *EFSA J.* 1300, 1–7. doi: 10.2903/j.efsa.2007.130r
- European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J.* 14:4634. doi: 10.2903/j.efsa.2016.4634
- Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J., and Ricke, S. C. (2011). Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Appl. Environ. Microbiol.* 77, 4273–4279. doi: 10.1128/AEM.00598-11
- Folster, J. P., Pecic, G., Bolcen, S., Theobald, L., Hise, K., and Carattoli, A. (2010). Characterization of extended-spectrum cephalosporin – resistant *Salmonella enterica* serovar heidelberg isolated from humans in the United States. *Foodborne Pathog. Dis.* 7, 181–187. doi: 10.1089/fpd.2009.0376
- Folster, J. P., Pecic, G., Singh, A., Duval, B., Rickert, R., Ayers, S., et al. (2012). Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar heidelberg isolated from food animals, retail meat, and humans in the United States 2009. *Foodborne Pathog. Dis.* 9, 638–645. doi: 10.1089/fpd.2012.1130
- Hendriksen, R. S., Vieira, A. R., Karlsmose, S., Lo Fo Wong, D. M. A., Jensen, A. B., Wegener, H. C., et al. (2011). Global monitoring of *Salmonella* serovar distribution from the world health organization global foodborne infections network country data bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathog. Dis.* 8, 887–900. doi: 10.1089/fpd.2010.0787
- Hopkins, K. L., Deheer-Graham, A., and Threlfall, E. J. (2006). Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum β -lactamase (CTX-M-40) isolated in the UK. *Int. J. Antimicrob. Agents* 27, 570–572. doi: 10.1016/j.ijantimicag.2006.03.003
- Jarquín, C., Alvarez, D., Morales, O., Morales, A. J., López, B., Donado, P., et al. (2015). *Salmonella* on raw poultry in retail markets in Guatemala: levels, antibiotic susceptibility, and serovar distribution. *J. Food Prot.* 78, 1642–1650. doi: 10.4315/0362-028X.JFP-15-117
- Jiang, X., Zhang, Z., Li, M., Zhou, D., Ruan, F., and Lu, Y. (2006). Detection of extended-spectrum β -lactamases in clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 50, 2990–2995. doi: 10.1128/AAC.01511-05
- Larsen, M. V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R. L., et al. (2012). Multilocus sequence typing of total-genome-sequenced bacteria. *J. Clin. Microbiol.* 50, 1355–1361. doi: 10.1128/JCM.06094-11
- Liakopoulos, A., Geurts, Y., Dierikx, C. M., Brouwer, M. S. M., Kant, A., Wit, B., et al. (2016). Extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg strains, the Netherlands. *Emerg. Infect. Dis.* 22, 1257–1261. doi: 10.3201/eid2207.151377
- Mevius, D. J., Dierikx, C. M., Veldman, K. T., Wit, B., van Pelt, W., and Heederik, D. (2015). *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2014 (MARAN)*. Available online at: https://www.wur.nl/upload_mm/e/0/d/104f4c0c-1126-4b64-b55b-80bed717802a_NethmapMaran2015.pdf
- Mughini-Gras, L., Enserink, R., Friesema, I., Heck, M., Van Duynhoven, Y., and Van Pelt, W. (2014). Risk factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg laying hens: a combined case-control and source attribution analysis. *PLoS ONE* 9:e87933. doi: 10.1371/journal.pone.0087933
- Nadon, C., Van Walle, I., Gerner-Smidt, P., Campos, J., Chinen, I., Concepcion-Acevedo, J., et al. (2017). Pulsenet international: vision for the implementation of whole genome sequencing (WGS) for global foodborne disease surveillance. *Eur. Surveill.* 22:30544. doi: 10.2807/1560-7917.ES.2017.22.23.30544
- Paauw, A., Fluit, A. C., Verhoef, J., and Leverstein-Van Hall, M. A. (2006). *Enterobacter cloacae* outbreak and emergence of quinolone resistance gene in Dutch Hospital. *Emerg. Infect. Dis.* 12, 807–812. doi: 10.3201/eid1205.050910
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., and Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 25, 1043–1055. doi: 10.1101/gr.186072.114
- Price, M. N., Dehal, P. S., and Arkin, A. P. (2010). FastTree 2 - Approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5:e9490. doi: 10.1371/journal.pone.0009490
- Public Health Agency of Canada (2016). Canadian Antimicrobial Resistance Surveillance System Report 2016. Available online at: <https://www.canada.ca/en/public-health/services/publications/drugs-health-products/canadian-antimicrobial-resistance-surveillance-system-report-2016.html>
- Rodríguez, C. E., Díaz-guevara, P., Moreno, J., Bautista, A., Montano, L., Realpe, M. E., et al. (2017). Laboratory surveillance of *Salmonella enterica* from human clinical cases in Colombia 2005 – 2011. *Enferm. Infecc. Microbiol. Clin.* 35, 417–425. doi: 10.1016/j.eimc.2016.02.023
- Siguier, P. (2006). ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res.* 34, D32–D36. doi: 10.1093/nar/gkj014
- Smith, H., Bossers, A., Harders, F., Wu, G., Woodford, N., Schwarz, S., et al. (2015). Characterization of epidemic Inc11-1 γ plasmids harboring ambl class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. *Antimicrob. Agents Chemother.* 59, 5357–5365. doi: 10.1128/AAC.05006-14
- Treangen, T. J., Ondov, B. D., Koren, S., and Phillippy, A. M. (2014). The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol.* 15:524. doi: 10.1186/s13059-014-0524-x
- United States Department of Agriculture (USDA) - Food Safety and Inspection Service (FSIS) (2013). Serotypes Profile of *Salmonella* Isolates from Meat and Poultry Products January 1998 through December 2013. Available online at: <https://www.fsis.usda.gov/wps/wcm/connect/c7b5903c-8e8b-4f85-9b5c-12eaf990d2dd/Salmonella-Serotype-Annual-2013.pdf?MOD=AJPERES>
- van Pelt, W., van der Zee, H., Wannet, W. J., van de Giessen, A. W., Mevius, D. J., Bolder, N. M., et al. (2003). Explosive increase of *Salmonella* Java in poultry in the Netherlands: consequences for public health. *Eur. Surveill.* 8, 31–35. doi: 10.2807/esm.08.02.00398-en
- Veldman, K. T., Mevius, D. J., Wit, B., van Pelt, W., and Heederik, D. (2016). *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2015 (MARAN)*. Available online at: [https://www.wur.nl/upload_mm/0/b/c/433ca2d5-c97f-4aa1-ad34-a45ad522df95_92416_008804_NethmapMaran2016\\$+TG2.pdf](https://www.wur.nl/upload_mm/0/b/c/433ca2d5-c97f-4aa1-ad34-a45ad522df95_92416_008804_NethmapMaran2016$+TG2.pdf)
- Voets, G. M., Fluit, A. C., Scharringa, J., Cohen Stuart, J., and Leverstein-Van Hall, M. A. (2011). A set of multiplex PCRs for genotypic

- detection of extended-spectrum β -lactamases, carbapenemases, plasmid-mediated AmpC β -lactamases and OXA β -lactamases. *Int. J. Antimicrob. Agents* 37, 356–359. doi: 10.1016/j.ijantimicag.2011.01.005
- Wagenaar, J. A., Hendriksen, R. S., and Carrique-Mas, J. (2013). Practical considerations of surveillance of *Salmonella* serovars other than Enteritidis and Typhimurium. *Rev. Sci. Tech.* 32, 509–519. doi: 10.20506/rst.32.2.2244
- Yoshida, C. E., Kruczkiewicz, P., Laing, C. R., Lingohr, E. J., Gannon, V. P. J., Nash, J. H. E., et al. (2016). The *Salmonella In Silico* Typing Resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. *PLoS ONE* 11:e0147101. doi: 10.1371/journal.pone.0147101
- Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., et al. (2012). Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 67, 2640–2644. doi: 10.1093/jac/dks261
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