International Journal of Food Microbiology 299 (2019) 1–7

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Occurrence, genotypes and antimicrobial susceptibility of *Salmonella* collected from the broiler production chain within an integrated poultry company

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ARTICLE INFO

Keywords:
S. Infantis
PFGE
$bla_{\text{CTX-M-65}}$
$bla_{\text{CTX-M-14}}$
Cross-contamination

ABSTRACT

Salmonella is a common foodborne pathogen in the poultry production systems. Its presence in this food industry is determined by the fact that it can survive and pass throughout the different steps in the poultry production. In this study we aimed to study the occurrence, genotypes and antimicrobial resistance of *Salmonella* collected from the broiler production chain within an integrated poultry company.

Three hundred fourteen samples were collected in the feeding plant, farms and the slaughterhouse. Samples were cultured for *Salmonella* isolation according to the ISO6579/Amd 1. Isolates were further typed by Kauffmann-White scheme and pulse field gel electrophoresis (PFGE). Antimicrobial resistance to 11 antimicrobials was studied by disk diffusion tests and sequencing of ESBL genes.

From the collected samples 70 (22%) were found to be *Salmonella* positive. The lowest *Salmonella* rates were found in feed samples while in farm and slaughterhouse samples *Salmonella* presence ranged from 5% to 88%. *S.* Infantis was the most common serotype (94%, 66/70). PFGE demonstrated that isolates belonged to 11 genotypes. Some genotypes were continuously identified throughout the production chain. 97% of the isolates showed resistance to at least one antimicrobial. Moreover, all *S.* Infantis isolates and one auto-agglutinable isolate showed resistance to at least 6 antimicrobials. 30 and 8 isolates were positive to $bla_{\text{CTX-M-65}}$ and $bla_{\text{CTX-M-14}}$ genes respectively. No bla_{KPC} resistance genes were identified in any isolate.

This study highlights the predominance of *S.* Infantis in the integrated poultry company. Genotypes showed that cross-contamination between stages of poultry production can occur, stressing the importance of implementing good hygiene practices in every level of the production. Moreover, multidrug resistance patterns and the presence of important ESBL genes have public health implications that need to be deeply discussed with a one health approach.

1. Introduction

Bacteria of the genus *Salmonella* are gram-negative rods which are widely distributed in food-producing animals. *Salmonella* contains more than 2500 serovars of which most are pathogenic for humans and might be transmitted by food (Barrow and Methner, 2013; OIE, 2008). Non-typhoidal salmonellosis is mostly acquired from food of animal origin (Bula-Rudas et al., 2015) and is characterized by fever, abdominal pain, and inflammatory diarrhea (WHO, 2016). Non-typhoidal *Salmonella* is estimated to be involved in almost 1 on 10 cases of human gastroenteritis and cause 420.000 deaths worldwide every year (WHO, 2015). The consumption of poultry products is one of the main causes of non-typhoidal salmonellosis (Barrow and Methner, 2013).

Moreover, antimicrobial resistant *Salmonella* is a serious concern around the world (WHO, 2016). The frequent presence of this pathogen in poultry production has been linked to the misuse of antibiotics in this sector (White et al., 2001). The latter is especially the case in developing countries where antibiotics are not only used for the treatment of infections but also as growth promoters and prophylactics in food-producing animals (Vinueza, 2017).

Several studies in developed countries have assessed the presence and characterized non-typhoidal *Salmonella* in different stages of the broiler production chain (Dias et al., 2016; Kim et al., 2015; Maurischat et al., 2014; Thung et al., 2016).

It must be considered that in many countries a part of the broiler production chain is owned by integrated companies which have not

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https://doi.org/10.1016/j.ijfoodmicro.2019.03.014
Received 3 August 2018; Received in revised form 8 March 2019; Accepted 22 March 2019

only broiler farms but also feed plants, breeders, slaughterhouses and in some cases supermarkets. This is the case in Ecuador, where 90% of poultry production is in the hands of integrated companies (Vinueza, 2017). Additionally, in Ecuadorian poultry production systems, antimicrobials are commonly used as growth promotors, prophylactics and therapeutics. This kind of practices can exercise a selective pressure for antimicrobial resistant *Salmonella* of public health concern (Wegener, 2012).

In Ecuador 1893 cases of foodborne salmonellosis were reported in 2016 (MSP, 2017) However little is known about the contribution of poultry meat production to these reported cases in humans. The aim of this study was to identify and characterize the diversity and antimicrobial resistance of *Salmonella* isolated from different production stages within an integrated poultry company.

2. Materials and methods

2.1. Study design and sampling

Salmonella detection was conducted on samples collected from three production stages within one integrated poultry company: feed production, broiler farms and slaughterhouse. Stages and types of collected samples are listed in Table 1.

In the feed plant samples of about 300 g of raw materials such as poultry meat meal ($n = 50$), hydrolyzed fish meal ($n = 10$), rice ($n = 30$), wheat bran ($n = 20$), palm kernel cake ($n = 17$), soy (n = 10), corn (10/177) and rice meal (n = 30); and compound broiler feed were aseptically collected. Samples were selected randomly from silos and storage bags. During the rearing period of the sampled flocks. One sample from every new batch of raw materials was collected.

Five poultry farms were included in this study. Every poultry farm was located on a different place. Each poultry farm contained 4 to 5 groups of houses which were separated from each other by a distance of 100 m up to 500 m. Every group of houses had 2 up to 5 houses and every house was considered to be a flock. One flock per group of houses was selected for sampling. In total, 21 flocks were followed. In order to sample one-day-old chicks from selected flocks, 40 papers from transport boxes were collected and subsequently pieces of these papers containing feces were pooled. To study the *Salmonella* status of each flock at the age of 30-days, three pairs of overshoes per flock were collected by walking four times along the stable housing the selected flock. A flock was considered positive when at least one pair of overshoes was positive for *Salmonella*.

The first tested flock per farm was selected for the follow-up at the slaughterhouse level. All flocks were slaughtered in the same slaughterhouse at the age of 42 days and each time as the first flock of the day. The slaughterhouse had a semi-automated slaughtering system (manual re-hanging and manual evisceration). During evisceration of each flock one caecum from 25 randomly selected chickens were collected. After final washing and after chilling in immersion water 5 breast skin samples from every selected flock were aseptically collected. Each sample consisted of the breast skin from 3 carcasses in order to collect at least 25 g.

Table 1
Stages and types of samples collected within the integrated poultry company.

Sampling stage	Type of sample	Number of samples
Feed production plant	Raw feed materials	177
	Compound broiler feed	10
Farms	Transport paper	21
	Overshoes	51*
Slaughterhouse	Caeca	5
	Skin after final washing**	25
	Skin after chilling	25

* Due to technical problems, 30-days old broilers from farm 4 could not be sampled. Therefore, 17 flocks were sampled.

** Final washing was performed after evisceration and before chilling in immersion water.

All samples were transported in an ice box within 1 h to the laboratory for bacteriological analysis.

Isolation and identification of *Salmonella*

From each feed sample, 25 g were weighted in a sterile plastic bag. From each of the 25 ceca/flock one gram was aseptically collected and pooled in order to have a sample of 25 g for analysis. From all pooled skin samples collected in the slaughterhouse, 25 g was weighted.

Salmonella isolation from all samples was carried out by a method based on the ISO 6579-1:2007 protocol (ISO, 2007). Briefly, to all samples 225 ml of Buffered Peptone Water (BPW; Difco, BD, Sparks, MD) was added, stirred vigorously and incubated at 37 °C for 20 h. Then, 100 μl of each broth was inoculated onto Modified Semi-solid Rappaport-Vassiliadis agar (MSRV; Oxoid, Basingstoke, UK) in three equidistant points. After incubation at 42 °C for 24 h all agar plates were examined for the presence of mobile bacteria recognizable by the presence of a white halo around one or more inoculation points on the medium. A loopful of this culture was taken from the edge of the white halo and streaked on a Xylose Lysine Deoxycholate agar (XLD, Difco) and incubated at 37 °C for 24 h. After incubation, one suspect colony for *Salmonella* was biochemically confirmed by Triple Sugar Iron (TSI, Difco, BD), Iron Lysine (LIA, BBL, BD), Urea (BBL, BD) and Sulfur Indole Motility tests (SIM, BBL, BD).

2.2. Salmonella serotyping

In order to limit the number of strains to be serotyped, isolates were grouped by an Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR as described by (Rasschaert et al., 2005). The ERIC PCR of the 70 tested strains was conducted within the same run. With ERIC PCR 33 different profiles were obtained. From each profile one isolate was selected for serotyping according to the Kauffmann-White scheme (WHOCC-Salm, 2007).

2.3. Genotyping of Salmonella isolates

In order to genotype the *Salmonella* isolates pulse field gel electrophoresis (PFGE) was carried out after digestion with *Xba*I enzyme according to the protocol described by PulseNet (CDC, 2013). Bands were analyzed using BioNumerics software V.7.6 (Applied Maths, Sint-Martems-Latem, Belgium). Fragments between 35 kb and 1140 kb in size were included in the analysis. The unweighted pair group method using arithmetic averages algorithm (UPGMA) with a 1% of tolerance was used to construct a dendrogram.

2.4. Antimicrobial susceptibility tests

All *Salmonella* strains were tested by the disk diffusion method. Breakpoints from the Clinical and Laboratory Standards Institute (CLSI, 2015) were used to determine antimicrobial resistance. Antimicrobials used were: gentamicin, nalidixic acid, ampicillin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline and kanamycin. Resistance patterns were determined by taking into account any non-susceptible (R + I) phenotype.

2.5. Detection of beta-lactamase genes

To perform PCR, bacterial DNA was extracted using the boiling method described by Parvej et al. (2016) with few modifications. Briefly, a pure bacterial colony was suspended and mixed in 300 μl of distilled water, boiled at 95 °C for 20 min. and centrifuged at 13,000 rpm for 3 min. The supernatant was collected and used as DNA template for PCR.

The presence of $bla_{\mathrm{CTX\text{-}M}}$, bla_{TEM}, bla_{CMY}, bla_{SHV} and bla_{KPC} genes was tested by PCR in isolates with phenotypic resistance to cefotaxime. PCR conditions and primers were the ones described by Hasman et al. (2005) for *bla*$_{\text{CTX-M}}$, Olesen et al. (2004) for *bla*$_{\text{TEM}}$, Kruger et al. (2004) for *bla*$_{\text{CMY}}$, Arlet et al. (1997) for *bla*$_{\text{SHV}}$ and Woodford et al. (2004) for *bla*$_{\text{KPC}}$. Sub-families of *bla*$_{\text{CTX-M}}$ genes were identified with PCR protocols described by Carattoli et al. (2008) for *bla*$_{\text{CTX-M-1}}$, Jiang et al. (2006) for *bla*$_{\text{CTX-M-2}}$, Hopkins et al. (2006) for *bla*$_{\text{CTX-M-8}}$, Paauw et al. (2006) for *bla*$_{\text{CTX-M-9}}$ and Dierikx et al. (2012) for *bla*$_{\text{CTX-M-14-like}}$. Amplification products were confirmed by gel electrophoresis using a 2% agarose gel. All PCR products were purified and sequenced at Macrogen Inc. (Seul-South Korea). Obtained sequences were aligned against reference sequences with the online tool ResFinder 2.1 (Zankari et al., 2012)

3. Results

3.1. Salmonella detection

From the 314 collected samples, 70 (22%) were found to be *Salmonella* positive. The lowest *Salmonella* rates were found in broiler feed samples while in farm and slaughterhouse samples *Salmonella* presence ranged from 5% (transport paper) to 88% (skin after final washing).

Only one sample (rice meal) of raw feed materials (0.5%, 1/177) was positive for *Salmonella*, while none of the 10 compound broiler feed samples were positive.

At the farm level, the transport papers (5%, 1/21) from only one flock (farm C, flock 2) was found positive for *Salmonella* while at the age of 30 days 13 flocks (77%, 13/17) were identified as positive based on the analysis of overshoes (Table 2). Within the positive flocks the number of positive overshoes ranged between 1 and 3.

From each farm the first tested flock was followed in the same slaughterhouse and was slaughtered as the first flock of the day. Based on the analysis of the ceca collected during the evisceration, the flock from farm A and E was *Salmonella* negative. Also, the flock from farm D was negative at slaughter. After final washing carcasses from each flock were *Salmonella* positive resulting in 22 of the 25 (88%) positive samples. After chilling carcasses from 4 flocks were positive, yielding 14 of the 25 (56%) samples positive.

S. Infantis was isolated from all stages and had the highest isolation rate (94%, 66/70). In contrast, *S.* Mbandaka (1%, 1/70) and *S.* Amsterdam (3%, 2/70) were only isolated from raw feed materials and overshoes (flock 1, farm C) respectively. One isolate from overshoes (flock 3, farm A) was auto-agglutinable.

Genotyping of *Salmonella* isolates

All 70 isolates in this study were subjected to PFGE analysis. In total 11 genotypes (Research Data) were identified. The 2 *S.* Amsterdam isolates belonged to the same genotype. Eight genotypes (I to VIII) were found within the group of the *S.* Infantis isolates. At farm level, some genotypes were present in different flocks reared on the same farm or in flocks of different farms (genotypes I, II, III and VI). Genotype I and VII were only found in one flock from farms E and B respectively.

Table 3

Genotypes of *S.* Infantis isolates.

Farms	Flock	Transport papers	Overshoes	Caeca	Carcasses after final washing	Carcasses after chilling
A	1		II (1/3) III (1/3) VIII (1/3)		II (2/5) III (3/5)	II (2/4) III (2/4)
	2		III (2/3) VIII (1/3)			
	3		I (1/2) VIII (1/2)			
B	1		VI (1/1)	III (1/1)	III (5/5)	
	2		III (3/3)			
	4		III (1/3) VI (1/3) VII (1/3)			
	5		III (2/3) VI (1/3)			
C	1		II (1/1)	II (1/1)	II (3/3)	III (2/3) IV (1/3)
	2	II (1/1)				
	3		II (2/2)			
	4		II (2/2)			
D	1				III (2/5) IV (2/5) V (1/5)	III (5/5)
E	1		I (1/1)		II (4/4)	II (2/2)
	2		III (1/1)			
	3		II (1/2) III (1/2)			

Some genotypes (I, VI, VII, VIII) were isolated exclusively from overshoes. Genotype II was the most spread one and was found in transport paper, overshoes, caeca samples and skin samples (before and after chilling) of flocks coming from different farms with exception of farm B.

Genotype III was present in overshoes, caeca samples and skin samples (before and after chilling) coming from different farms. Genotypes IV and V were only present in skin before chilling from flocks of farm D (Table 3). The isolates belonging to the 3 other *Salmonella* serotypes (auto-agglutinable *Salmonella*, *S.* Mbandaka and *S.* Amsterdam) corresponded to genotype IX, X and XI respectively.

3.2. Antibiotic susceptibility testing and detection of resistance genes

The disc diffusion method delivered 68 (97%) isolates which showed resistance to at least one antimicrobial. Two isolates (the *S.*

Table 2

Salmonella in samples collected at the farm and the slaughterhouse level.

Farm	Farm level*																				Slaughterhouse level**										
	Transport paper					Overshoes															Caeca	Skin after final washing					Skin after chilling				
	1	2	3	4	5	1			2			3			4			5			1	1	2	3	4	5	1	2	3	4	5
A	-	-	-	-		+	+	+	+	+	+	+	+	+	-	-	-				-	+	+	+	+	+	+	+	+	-	+
B	-	-	-	-	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
C	-	+	-	-		+	+	+	-	-	-	-	+	+	+	+	-				+	-	+	+	+	-	+	-	+	-	+
D	-	-	-	-		Not sampled															-	+	+	+	+	+	+	+	+	+	+
E	-	-	-	-		+	-	-	-	-	+	+	-	+	-	-	-				-	+	+	-	+	+	-	-	+	+	-

Table 4
Non-susceptibility rates of *S.* Infantis isolates to tested antibiotics.

Antibiotic	Non-susceptibility rate[*]
Nalidixic acid	100%
Ciprofloxacin	100%
Cefotaxime	99%
Ampicillin	99%
Tetracycline	94%
Sulfamethoxazole + trimethoprim	92%
Chloramphenicol	88%
Kanamycin	80%
Gentamicin	74%
Ceftazidime	6%

[*] $n = 66$.

Table 5
Antimicrobial resistance patterns and origin of *Salmonella* isolates.

N° of classes of antimicrobials	Patterns	Number of isolates (%)	$bla_{\text{CTX-M-14}}$	$bla_{\text{CTX-M-65}}$	Origin
6	SGFNCDTHKA	4 (6)	1	1	o, s
6	SGFNCTHKA	[a]29 (41)	2	12	o, s
6	SFNCTHKA	15 (21)	3	6	o, s, c
6	SGFNCTHA	4 (6)	–	2	o, s
5	GFNCTHKA	1 (1)	–	–	s
5	SGFNCHKA	1 (1)	–	1	s
5	SGFNCTA	6 (9)	–	5	o, s, c
4	GFNCHKA	3 (4)	1	2	s
5	SGFNTHK	1 (1)	–	–	o
5	SFNCTHA	1 (1)	–	–	s
4	SFNCTA	1 (1)	1	–	t
4	GFNCTA	1 (1)	–	1	o
1	K	[b]1 (1)	–	–	o
0	N/A	[b,c]2 (3)	–	–	r, o
	Total	70 (100)	8	30	

Antibiotics: sulfamethoxazole + trimethoprim (S), gentamycin (G), ciprofloxacin (F), nalidixic acid (N), cefotaxime (C), ceftazidime (D), tetracycline (T), chloramphenicol (H), kanamycin (K), ampicillin (A). N/A: not applicable. Origin: raw feed materials (r), transport paper (t), overshoes (o), caeca (c), skin (s).

[a] Including the auto-agglutinable isolate.

[b] *S.* Amsterdam.

[c] *S.* Mbandaka.

Mbandaka isolate and one *S.* Amsterdam isolate) were fully susceptible to all tested antimicrobials, while the other *S.* Amsterdam isolate was only resistant to kanamycin. The 66 *S.* Infantis isolates presented a low resistance rate for ceftazidime (6%), but high resistance rates for the other tested antimicrobials ranging from 71% up to 96% (Table 4). All *S.* Infantis isolates and the auto-agglutinable isolate showed resistance to at least 6 antimicrobials. Patterns 2 and 3 were the most common ones (41% and 21% respectively) harboring 7 groups of antibiotics (Table 5).

The only group of genes identified in isolates with cephalosporin resistant phenotypes was $bla_{\text{CTX-M}}$ (59%; 39/66). Within this group, 30 isolates belonged to $bla_{\text{CTX-M-65}}$ and 8 isolates to $bla_{\text{CTX-M-14}}$ (Table 5). One isolate (type 2) could not be amplified with $bla_{\text{CTX-M}}$ sub-family PCR, bla_{KPC} resistance gene was not identified in any isolate.

Isolates above dashed line have multidrug resistant phenotypes.

4. Discussion

In this study we aimed to determine the presence and AMR patterns of *Salmonella* at different stages within the broilers production chain.

From the feed production plant *Salmonella* was isolated from 1% of the samples. Low prevalence of *Salmonella* in poultry feed has also been reported in other studies carried out in Latin America. For example, a study carried out in Ecuador reported that 4% of raw materials for poultry feed were *Salmonella* positive to (Villagómez et al., 2016). Similarly, 3% of poultry feed samples were found positive in Brazil (Pellegrini et al., 2015). Concordantly, studies in developed countries have shown contamination rates that range from 4.7 to 12% (Jones and Richardson, 2004; Torres et al., 2011; Whyte et al., 2003). Low contamination rates in poultry feed can be explained by the lack of homogeneity of the *Salmonella* contamination in large feed volumes (Jones, 2011) leading to the underestimation of its presence in such products. Another factor that can influence the limited detection of *Salmonella* in poultry feed could be the low water activity of grains and poultry feed, which reduces growth possibilities of *Salmonella* when present (ICMSF, 2011).

In order to evaluate the presence of *Salmonella* in one-day-old chicks, transport papers were analyzed. Our results show that 5% of these samples were positive for *Salmonella*. This result differs from other studies performed in Brazil, Belgium, and Spain where *Salmonella* was reported in 11% to 50% of transport paper samples (Heyndrickx et al., 2002; Rocha et al., 2003; Marin et al., 2011). On the other hand, within the European Union *Salmonella* prevalence in one-day-old chicks was 0.3% in 2016 (EFSA and ECDC, 2017). Presence of *Salmonella* in one-day-old chicks has been linked to contamination of broiler breeders (Fris and Van Den Bos, 1995; Limawongpranee et al., 1999). It must be considered that in Ecuador, disinfection of eggs prior incubation (immersion into allowed disinfectants) and periodic disinfection of hatcheries (with detergents and disinfectants) is a common practice in integrated companies. These practices can explain the low detection rate of *Salmonella* at this step. Low prevalence of *Salmonella* in breeder flocks could have also influenced the results. However further studies are necessary to determine the role of breeder flocks in the transmission of *Salmonella*.

Salmonella status at the farm level showed that 13/17(77%) of flocks were positive for *Salmonella*. These results are similar to studies in Colombia (65%; $n = 315$) and Peru (49%; $n = 132$) (Donado-Godoy et al., 2012; Valderrama et al., 2007). Berghaus et al. (2013) even demonstrated that 90.9% of sampled farms in the United States ($n = 50$) were positive for *Salmonella*. On the other hand, for the European countries a *Salmonella* prevalence at flock level of 2.6% was reported in 2016 (EFSA and ECDC, 2017).

In other Latin American countries, low *Salmonella* prevalence at farm level has also been reported: studies in Brazil and Venezuela reported 5% ($n = 40$) and 23% ($n = 332$) contaminated farms respectively (Boscán-Duque et al., 2007; Giombelli and Gloria, 2014). Risk factors determining *Salmonella* infection of broilers on farms have been related to defects in biosecurity protocols (Rose et al., 2000; Valderrama et al., 2007). The open configuration of Ecuadorian poultry houses may lead to gaps in biosecurity resulting in *Salmonella* colonization of birds.

From each flock followed during slaughter, caeca were collected for testing the presence of *Salmonella*. Testing caeca content at slaughterhouse, only 2/5 (40%) of the flocks were *Salmonella* positive; while testing at the farm all 4 tested flocks were positive. This difference may be due to the fact that the content of only 25 caeca were examined. This number of caeca allowed to detect flocks with a *Salmonella* prevalence rate of at least 11% within the flock (considering a flock of 5000 birds and a 95% confidence level) indicating that the chance for the detection of contaminated flocks with a lower prevalence will be largely reduced (de Blas, 2006).

Salmonella was present in 88% of the skin samples after final washing and 56% of the skin samples after chilling. Villagómez et al. (Villagómez et al., 2016) in Ecuador obtained similar results at this level, reporting *Salmonella* in 79% of skin samples before and after chilling. Presence of *Salmonella* on carcasses after slaughter can be related to cross-contamination during steps of the slaughter processes like evisceration and/or slaughter environment (Olsen et al., 2003; Rasschaert et al., 2007).

In this integration, *S.* Infantis was the predominant serotype which is in concordance with our previous study where 388 flocks were tested for *Salmonella* (Vinueza-Burgos et al., 2016). In that study, 84% (52/62) of *Salmonella* isolates were serotyped as *S.* Infantis. A high prevalence of *S.* Infantis (90%) in poultry has also been reported in Peru (SENASA, 2015). On the other hand, other Latin American countries have reported other serotypes as the most prevalent. For instance, *S.* Paratyphi B dT+ and *S.* Paratyphi B have been reported as predominant serotypes in Colombia and Venezuela respectively (Boscán-Duque et al., 2007; Donado-Godoy et al., 2014), while in Brazil *S.* Infantis is ranked as the 2nd in poultry related serotypes with a prevalence of 8% (Medeiros et al., 2011). The close interactions of the poultry industry between Ecuador and Peru could be a determining factor for the common predominance of *S.* Infantis in the poultry industry of these countries.

Different *S.* Infantis genotypes found in this study are widespread in different stages of the integrated company. Analysis of genetic types indicates possible sources of contamination of broilers at the farm and carcasses at the slaughterhouse level. This fact can be observed when looking at the genotype II that could be originated from feces of one-day old chicks and appeared along the production chain of all studied flocks with exception of flock D. Other genotypes as IV and V are only found on carcasses, highlighting the possible consequences of a deficient cleaning and disinfection process leading to cross contamination of carcasses by the environment during slaughter.

These findings can support the fact that *Salmonella* genotypes can move within the integration due to failures in biosecurity procedures as has been demonstrated elsewhere (Lee et al., 2007; Voss-Rech et al., 2015). Biosecurity gaps can also provoke these genotypes to reach other integrated companies as was observed by Vinueza-Burgos et al. (2016) who reported that common *Salmonella* genotypes were found in farms belonging to 6 integrations in Ecuador. Of special relevance, cross-contamination may occur at the slaughterhouse level. This could imply that the slaughter of negative flocks results in the delivery of positive carcasses contaminated with strains coming from previous positive flocks (Dias et al., 2016). Although some genotypes appear to be lost in the final stages of production, it should be considered that less prevalent genotypes could not be recuperated due to the limited number of analyzed samples. These observations highlight the importance of a continue monitoring of *Salmonella* genotypes to have a broader view of *Salmonella*'s dynamics in an integrated poultry company.

All *S.* Infantis isolates in our study (94%) presented multidrug resistance patterns. Multiresistance in *S.* Infantis isolates from commercial poultry in Ecuador has been reported in our previous study (Vinueza-Burgos et al., 2016). This latter research showed that *S.* Enteritidis isolates were mostly susceptible, highlighting the importance of the analysis of antimicrobial resistance based on specific serotypes.

Studies on *Salmonella* resistance at farm level in other Latin American countries are limited. However, it has been previously reported that *Salmonella* serotypes (eg. *S.* Parathyphi B, *S.* Heidelberg, *S.* Enteritidis and *S.* Infantis, *S.* Typhimurium) isolated from poultry and poultry meat products presented high rates of multi-drug resistant phenotypes (Boscán-Duque et al., 2007; Medeiros et al., 2011; Quesada et al., 2016; Rodriguez et al., 2015).

It should be noticed that *S.* Amsterdam and *S.* Mbandaka isolates had low resistance profiles which is in accordance to other studies carried out on these serotypes (Parveen et al., 2007; Ziyate et al., 2016). A previous study in Ecuador also concludes that other serotypes than *S.* Infantis were rather susceptible (Villagómez et al., 2016).

Only *bla*$_{\text{CTX-M}}$ genes from group 9 were found in cephalosporin resistant phenotypes. Sequencing of these genes demonstrated that *bla*$_{\text{CTX-M-65}}$ (77%) followed by *bla*$_{\text{CTX-M-14}}$ (21%) were the most frequent ones. These genes have also been reported in *S. Indiana, S.* Typhimurium and *S.* Enteritidis isolated from broilers in China (Li et al., 2014; Zhang et al., 2016). In this country, *S. Indiana* harboring *bla*$_{\text{CTX-M-65}}$ has also been isolated from poultry and human samples (Bai et al., 2016). Moreover, Franco et al. (2015) reported the presence of poultry associated *S.* Infantis carrying *bla*$_{\text{CTX-M-65}}$ isolated from a human sample in Italy. Additionally, in the USA it has been recently reported that *S.* Infantis carrying *bla*$_{\text{CTX-M-65}}$ are present in isolates from poultry farms, retail chickens and humans indicating a possible epidemiological association among those strains (Tate et al., 2017). In Ecuador, *bla*$_{\text{CTX-M-65}}$ positive *S.* Infantis isolated from humans has been recently described (Cartelle Gestal et al., 2016). These findings suggest that *bla*$_{\text{CTX-M-65}}$ gene in *S.* Infantis may circulate in Ecuador and the surrounding region. More research is needed at regional level to better understand the epidemiology of resistance genes in *Salmonella* of poultry origin and their implication in public health.

In conclusion, this paper shows that *Salmonella* contamination in a broiler production chain can start in the early stages of the system and maintain over the time. More research on *Salmonella* in the different stages of poultry integrations is of great interest in order to better understand the dynamics of this bacteria in the food production process. Based on these results efficient control programs that consider effective hygienic measures could be developed. High antimicrobial resistance is also of concern if these pathogens reach the final consumers. An active surveillance of antimicrobial resistance in *Salmonella* is recommended at national and regional level.

Acknowledgments

We acknowledge the integrated poultry company that participated in this study and Ximena Sanchez and Cristina Logacho for sample collection and laboratory work. We acknowledge for the financial suport of this research to Académie de Recherche et d'Enseignement supérieur (ARES), grant 082-ARES-UG.

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