Investigating the dynamics of *Salmonella* contamination in integrated poultry companies using a Whole Genome Sequencing approach.

José Luis Medina Santana

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Members of the jury

Chairperson: Dr. Darshana Morar-Leather, University of Pretoria, Faculty of Veterinary Science, South Africa

External Member: Prof. Paul Tshepo Matjila, University of Pretoria, Faculty of Veterinary Science, South Africa

Internal Member:
Dr. Veronique Dermauw, Institute of Tropical Medicine, Department of Biomedical Sciences, Belgium

Readers
Dr. Pieter Monsieurs, Institute of Tropical Medicine, Department of Biomedical Sciences, Belgium
Dr. Amanda Beylefeld, University of Pretoria, Faculty of Veterinary Science, South Africa

Supervisors
Dr. Mieke Stevens, Institute of Tropical Medicine, Department of Biomedical Sciences, Belgium
Dr. Christian Vinueza, Central University of Ecuador, Faculty of Veterinary Medicine

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Summary

The study of non-typhoid *Salmonella* in broiler integrations has been limited by the resolution of typing techniques. Although serotyping of *Salmonella* isolates is used as a traditional approach, it is not of enough resolution to clearly understand the dynamics of this pathogen within poultry companies. The aim of this research was to investigate the epidemiology and population dynamics of *Salmonella* serotypes in two poultry integrations using a whole genome sequencing approach. Two hundred and forty-three *Salmonella* isolates recovered from the broiler production chain of two integrated poultry companies were whole genome sequenced and analyzed with dedicated databases and bioinformatic software. The analyses of sequences revealed that *S.* Infantis was the most frequent serotype (82.3%). Most isolates showed a potential for resistance against medically-important antibiotics and disinfectants. Furthermore, 97.5% of isolates harbored the pESI-like mega plasmid, that plays an important role in the global dissemination of AMR. SNP tree analysis showed that there were clones that are niche-specific while other ones were distributed throughout the broiler production chains. In this study we demonstrated the potential of whole genome sequencing analysis for a comprehensive understanding of *Salmonella* distribution in integrated poultry companies. Data obtained with these techniques allow determination of the presence of genetic factors that play an important role in the environmental fitness and pathogenicity of *Salmonella*.

Key words: *Salmonella*; WGS; antimicrobial resistance.

LIST OF ABBREVIATIONS

3GC	Third Generation Cephalosporins
AMR	Antimicrobial Resistance
cgMLST	Core genome MLST
DALIs	Disability Adjusted Life Years
DNA	Deoxyribonucleic acid
ESBL	Extended-spectrum beta-lactamase
GDAMR	Genetic determinants of antimicrobial resistance
MDR	Multidrug resistant
MIC	Minimal Inhibitory Concentration
MLST	Multilocus sequence typing
NTS	Non-typhoidal Salmonella
PCR	Polymerase Reaction Chain
pESI	Plasmid of Emerging Salmonella Infantis
PFGE	Pulsed-field gel electrophoresis
RAPD	Random amplification of polymorphic DNA
REP	Repetitive extragenic palindromic
SNP	Single nucleotide polymorphism
USA	United States of America
wgMLST	Whole-genome MLST
WHO	World Health Organization

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1. Introduction

Non-typhoidal *Salmonella* (NTS) *enterica* subsp. *enterica* is a foodborne pathogen that causes ~1.2 million infections in the United States of America (USA) annually (Scallan *et al.*, 2011), and was responsible for more than 95 million cases of diarrheal disease and 50,771 deaths worldwide in 2017 (Stanaway *et al.*, 2019). Human infections are usually mild and manifest as a self-limiting gastroenteritis. However, high-risk populations such as infants, the elderly, and immunocompromised individuals can develop systemic disease that requires antibiotic intervention (Crump *et al.*, 2015). Moreover, the rise of multidrug resistant (MDR) NST strains have resulted in infections that are unresponsive to first-line treatment, thus requiring more complicated options (Parry, 2003; Mølbak, 2005). Studies have indicated that the increased prevalence of MDR NTS results from the overuse of antibiotics in agriculture, veterinary and human medicine (Llor and Bjerrum, 2014; Ventola, 2015). Presumably, these practices have placed selection pressure on MDR NTS strains, leading to its clonal expansion, global dissemination, and persistence. NTS have a broad host range and occupy the gastrointestinal track of multiple species including mammals, reptiles and birds (Uzzau *et al.*, 2000; Mitchell and Shane, 2001; Editorial team *et al.*, 2008). Consequently, carrier species and their meat byproducts present the risk of NTS-mediated zoonosis and foodborne infections NTS (Braden, 2006). Several serotypes that are commonly isolated from human infections, are also prevalent in poultry production systems. Despite being well-linked to NTS infections in humans (Antunes *et al.*, 2016), poultry is one of the most widely consumed and affordable protein source in the world (Magdelaine, Spiess and Valceschini, 2008; Windhorst, 2017). A rising demand for this commodity has led to more intensive farming practices in order to meet consumer demands.

Intensive poultry farming involves increased stocking densities (more birds per unit floor space), faster growing breeds, and reduced downtime. Good sanitation practices are difficult to maintain under these conditions, thus enabling easier *Salmonella* transmission in these environments. Poultry meat production occurs in vertically integrated operations that consist of breeder farms, hatchery operation, a grow-out phase, slaughter and carcass processing (Glatz and Pym, 2015). NTS contamination can enter into any of these production steps. Moreover, NTS can contaminate the broilers production chain via contaminated feed, rodents, wild birds, or by other breaches in biosecurity (Cox *et al.*, 1983; Davies and Wales, 2010; Totton *et al.*, 2012). Multiple genetic factors that confer phenotypic traits such as adhesion capacity, resistance to sanitizers and heavy metals, as well as immune-evasion mechanisms allow for various serotypes of NTS to persist in the production environment and establish successful infections. Effective implementation of strategies to control NTS in integrated poultry companies is therefore difficult. Serotypes but also genotypes must be considered to understand the dynamics of NTS in such production systems.

Salmonella enterica serovar Infantis (*S.* Infantis) is an emergent serotype worldwide. This serotype, has been reported as one of the most prevalent NTS in humans in Europe and USA (EFSA and ECDC, 2019; Tack *et al.*, 2019). Besides, several studies describe *S.* Infantis as the most prevalent serotype in poultry (Valderrama *et al.*, 2014; Vinueza-Burgos *et al.*, 2016; EFSA and ECDC, 2019). Moreover, multidrug resistant phenotypes of this serotype are of public health concern. (Shah *et al.*, 2017; Mejía *et al.*, 2020; EFSA and ECDC, 2021).

Plasmids are the main mobile elements involved in *Salmonella* adaptation. Particularly, a rise in the incidence of *S.* Infantis was registered since 2003 worldwide. Interestingly, a large conjugative plasmid named plasmid of Emerging *Salmonella* Infantis or pESI whose structure contains determinants for resistance to various antibiotics was identified (Aviv *et al.*, 2014). Posteriorly, pESI-like plasmids have been described in *S.* Infantis worldwide, and their implication in the rise of *S.* Infantis has been suggested (Franco *et al.*, 2015; Aviv, Rahav and Gal-Mor, 2016; Tate *et al.*, 2017; Gymoese *et al.*, 2019; Alba *et al.*, 2020; Bogomazova *et al.*, 2020; Cohen, Rahav and Gal-Mor, 2020; García-Soto *et al.*, 2020; McMillan *et al.*, 2020; Mejía

et al., 2020; Tyson *et al.*, 2021; Kürekci *et al.*, 2021). Despite the relevance of these genetic elements, there is scarce information about pESI-like plasmids in the Andean region (Vallejos-Sánchez *et al.*, 2019; Burnett *et al.*, 2021).
The aim of this study was to investigate the epidemiology and population dynamics of *Salmonella* serotypes present within two poultry integrations, and to determine the presence of resistance and virulence genetic factors that may contribute to the environmental fitness and pathogenicity of NTS, using a whole genome sequencing approach.

2. Literature review

2.1. *Salmonella enterica*.

Salmonella enterica subsp. e*nterica* is a bacterium able to infect and colonize a wide range of hosts, humans among them. Several domestic and wild animals (poultry, cattle, swine, etc.) can be reservoirs, where the status of chronical *Salmonella* carrier is common. However, the status of asymptomatic chronic carrier is not common in humans, being most of the serotypes pathogenic for people (Braam, 2005). The disease caused by this pathogen is called salmonellosis and according to the World Health Organization (WHO), it is among the main fourth causes of diarrheal disease. Furthermore, the emergence of multi-resistant strains contaminating the food chain makes *Salmonella* a world public health concern (WHO, 2018). These bacteria have an oral fecal transmission and can be transmitted from wild and domestic animals to food derivatives (e.g. eggs and poultry meet) (Silva *et al.*, 2014). Even though salmonellosis is usually self-limiting, in susceptible population (children and elderly) hospitalization and antimicrobial treatment could be necessary (Levine and Powers, 2015).

2.1.1. Taxonomy and characteristics of non-typhoidal *Salmonella*.

The current taxonomy classifies *Salmonella* genus into two species: *Salmonella enterica* and *Salmonella bongori*. The same way, *Salmonella enterica* has been divided into six subspecies replacing the previous groups I, II, IIIa, IIIb, IV, and VI (WHOCC-Salm, 2007; Agbaje *et al.*, 2011). According to this classification the current groups are:

- *S. enterica* sub-specie *enterica* (previous group I),
- *S. enterica* sub-specie *alamae* (previous group II),
- *S. enterica* sub-specie *arizonae* (previous group IIIa),
- *S. enterica* sub-specie *diarizonae* (previous group IIIb),
- *S. enterica* sub-specie *indica* (previous group IV), and
- *S. enterica* sub-specie *houtenae* (previous group IV).

Furthermore, *S. enterica* sub-specie *enterica* (called *S. enterica* from here on) is a group with more than 2600 serotypes classified according to its antigenic structure (Sanderson and Nair, 2013). Many of them are pathogens of different animals' species, and almost all of them have the potential to be pathogens for humans (Eng *et al.*, 2015). According to this structure, *Salmonella* serotypes classification could result long and complicated. However, in practical life, the genus *Salmonella* is used followed by the serotype. Thus, *Salmonella enterica*, sub-specie *enterica* Serotype Infantis is written "*S.* Infantis", using a capital letter at the beginning without italic to empathize the serotype name (Tindall *et al.*, 2005).

A common and traditional way to classify this bacterium is based on pathogenicity to the human host. According to this perspective *S. enterica* can be divided in typhoidal and non-typhoidal *Salmonella*. The typhoidal *Salmonella* group include *S.* Typhi and *S.* Paratyphi. The diseases

produced by these serovars are called typhoid and paratyphoid fever respectably (Nuccio and Bäumler, 2014). A graphic representation of *Salmonella* classification with its principal serovars was described by Langridge, Wain and Nair, (2012) and can be appreciated below (Figure 1).

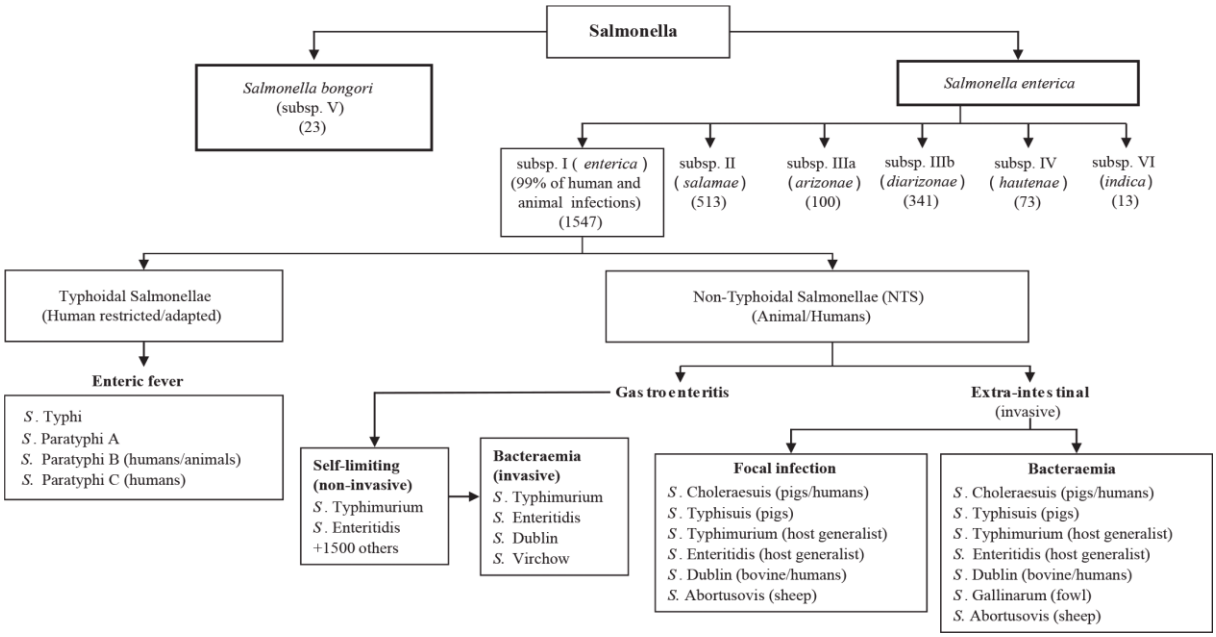


Figure 1. Classification diagram of genus *Salmonella*.
(1) Numbers in brackets indicate the total number of serotypes included in each subspecies. (2) Relevant serovars for clinical and husbandry are listed, but all have the potential to be pathogens for one or another species in suitable conditions. Source: Figure reproduced by Langridge, G.C., et al., 2008. Invasive Salmonellosis in humans. Chapter 8.6.2.2, and Kenneth et al., 2013. Taxonomy and Species Concepts in the Genus *Salmonella*.

Pathogenicity features of typhoidal *Salmonella* serovars allow them to overpass the gut and disseminate in blood. For this reason, these pathogens are associated with bacteremia febrile processes. On the other hand, non-typhoidal *Salmonella* serovars produce localized gastroenteritis, mainly associated with immunocompromised individuals (Nuccio and Bäumler, 2014).

S. Typhi is a pathogen limited to human hosts, while *S.* Paratyphi can colonize other animal species. However, both of them are high adapted to humans and can induce several diseases (Luby, 2014). Typhoidal diseases are characterized by an initial invasion of intestinal mucosa generally without immediate inflammatory response (diarrhea). This characteristic allows a quick dissemination and bacteremia of typhoidal *Salmonellas* before first symptoms appear (Dougan and Baker, 2014).

On the other hand, non-typhoidal *Salmonella*, are serotypes adapted to a great variety of warm blooded animals. In these hosts, exist high pathogenic serotypes for specific species like poultry (*S.* Gallinarum), pigs (*S.* Choleraesuis), and sheep (*S.* Abortusovis). However, most of them don't show a clinical presentation (Barrow and Methner, 2013). From wildlife to pets and livestock, these pathogens can be asymptomatically harbored in the gastrointestinal tract of several animals and be disseminated by feces. Therefore, typhoidal *Salmonella* serotypes can reach humans through foods contaminated during the production process. Besides, food handling, in the commercialization and consumption chain, and direct contact with fecal-contaminated environments can play an important role in the transition of the pathogen (Demirbilek, 2018).

Non-typhoidal salmonellosis in humans is characterized by gastrointestinal illness a few hours after the consumption of contaminated food. This symptom is the key factor to distinguish non-typhoidal salmonellosis from the disease caused by typhoidal *Salmonella* (Dougan and Baker, 2014). Non-typhoidal salmonellosis is one of the main causes of diarrhea worldwide, although invasive presentations like bacteremia, meningitis, and osteomyelitis can be also presented in immunocompromised persons (Wen, Best and Nourse, 2017).

2.1.2. Epidemiology.

Infections by different serotypes of typhoidal and not-typhoidal *Salmonella* are reported worldwide every year. Although the presentation rate of typhoid infections is less than non-typhoidal (around 10–20 million cases per year), its more severe and has higher fatality rates (100,000–200,000 deaths every year)(Johnson, Mylona and Frankel, 2018). On the other hand, the less severe symptoms of non-typhoidal salmonellosis have made this disease unappreciated, mainly in developing countries. However, non-typhoidal *Salmonella* is currently the second main cause of gastroenteritis, reaching more than 95 million cases of diarrheal disease and 50,771 deaths worldwide (Stanaway *et al.*, 2019).

Although non-typhoidal *Salmonella* is usually the second general cause of gastroenteritis, studies carried out in the USA and China place it as the main cause of enteric illness in infants (under 5 years of age)(Scallan *et al.*, 2013; Li *et al.*, 2014). This situation increases its severity in low-income countries where factors like anemia, malnutrition, HIV, and other tropical diseases increase the presentation of invasive non-typhoidal salmonellosis (Gilchrist and MacLennan, 2019).

Several sources of *Salmonella* contamination have been described in drinking water, sewage, birds, wild and domestic animals, reptiles, agricultural animals, and food (Murray, 1991). Water, for example, was responsible for an important *S.* Enteritidis outbreak in Croatia in 2014. Gastrointestinal symptoms were reported in 68 patients from the consumption of contaminated groundwater from a spring. (Kovačić, Huljev and Sušić, 2017). Another interesting case related to wild songbirds was described in the USA. This outbreak reported 29 illnesses and 14 hospitalizations in 12 states (CDC, 2021). In both cases, the real estimation of affected people reached hundreds more.

Besides, several studies worldwide have place squamates (lizards, snakes, and amphibians) as an important source of this pathogen. The increasing popularity of these animals as pets has risen the risk of *Salmonella* infection, especially in children. Thus, It is estimated that around 3.5% of general salmonellosis cases are linked to reptile exposition. (Whiley, Gardner and Ross, 2017).

A similar scenario can be appreciated with dogs and cats. These companion animals are the most common pets in the world and generally course the infection without apparently symptoms (Bataller *et al.*, 2020). An example was reported in 2019 when an outbreak involving pet food products caused 358 clinical human cases, 133 hospitalizations and 1 death in 42 states of the USA (CDC, 2019b).

Although all these pathways are important for *Salmonella* contamination, food of animal origin remains the main source of human salmonellosis (Plym-Forshell and Wierup, 2006). The fact that some serotypes can colonize the intestine and be harbored without symptoms in farm animals, may be an important route for human exposure. This pathway has been widely

documented in different animal industries like poultry and pig production (Evangelopoulou *et al.*, 2014; Antunes *et al.*, 2016; Bonardi, 2017).

Recent studies estimated the foodborne burden of *S.* enterica (Typhoidal and not-typhoidal serovars) in 21.2 million of Disability Adjusted Life Years (DALYs) for all transmission sources. Interestedly, 8.76 million of these DALYs were attributed to contaminated food (WHO, 2015b).

The most common non-typhoidal serotypes isolates from human infections are *S.* Enteritidis and *S.* Typhimurium witht around of 80% of reports (Wen, Best and Nourse, 2017; Ferrari *et al.*, 2019). However, *S.* Infantis has been gaining space as ethological agent of non-typhoidal salmonellosis (Tack *et al.*, 2019; Cohen, Rahav and Gal-Mor, 2020).

Currently, poultry is the main reservoir of *S.* Infantis. This bacterium goes unnoticed in the intestine of these animals, so its presence in chicken meat is a risk factor for the transmission of salmonellosis to humans (Kalaba *et al.*, 2017). The main routes of transmission of this pathogen are: 1) the consumption of undercooked chicken meat contaminated with this pathogen during the slaughter process, and 2) cross-contamination of other foods and kitchen implements during meals preparation (Finazzi *et al.*, 2019).

The emergent *S.* Infantis has been reported in Europe and the USA, mainly linked to poultry (Dar *et al.*, 2017; EFSA and ECDC, 2019). Outbreaks produced by this serotype have also been present in these countries (Basler *et al.*, 2016; CDC, 2019a; Finazzi *et al.*, 2019). Similarly, *S.* Infantis has been isolated from farms and poultry products in several South American countries, included Ecuador (Vinueza-Burgos *et al.*, 2016; Cunha-Neto *et al.*, 2018; Mejía, Vela and Zapata, 2021). However, reports of human cases have been less frequent and in a smaller scale (Almeida *et al.*, 2013; Cartelle Gestal *et al.*, 2016; Granda *et al.*, 2019).

This difference in the reports of human cases can be explained by the deficient or non-existent foodborne surveillant programs in these countries, in addition to socioeconomic factors (low-income, deficient health care service, etc.)(ISAGS, 2012). Besides, Latin America idiosyncrasy is another aspect to consider. Since the access to antibiotics is usually easy, many people prefer to self-medicate, producing *Salmonella* cases that remain undiagnosed. Despite of this fact, 1099 cases of non-typhoid salmonellosis were reported in Ecuador in 2020, and 516 more until August 2021 (MSP, 2021).

All these factors highlight the importance of *Salmonella* as a public health problem that needs to be controlled, especially in low and middle-income countries where most of the fatal cases are presented.

2.2. Antimicrobial resistance.

Antibiotics are chemicals compounds that either avoid bacterial replication or kill them. The first antibiotic was discovered by Alexander Fleming in 1928 and since then, antibiotics have saved millions of lives (Rang et al., 2008). However, the misuse of this products has promoted the selection of pathogenic resistance strains (Reygaert, 2018).

Antimicrobial resistance is the capacity that some bacteria have to avoid the action of antibiotics. The antimicrobial resistance mechanisms are the inactivation of drug, limitation of drug uptake, modification of the drug target, and the action of active efflux pumps that take out the drug. These resistance mechanisms can be classified according to their genetic origin in two groups, native or acquired (Reygaert, 2018).

Native resistance mechanisms are given by genes that are shared by all individuals within the same species and can be intrinsic or induced. Intrinsic resistance is a trait always expressed in the specie, while induced resistance is given by inactive genes naturally integrated in the genome of bacteria (Martinez, 2014). These inactive genes are only expressed after exposure to an specific antibiotic (Cox and Wright, 2013).

On the other hand, acquired resistance is acquired from other bacteria by different routes of genetic transfer (transformation, transposition, and conjugation) which can be temporary or permanent. The mutations in the chromosomal deoxyribonucleic acid (DNA) (Single Nucleotide Polymorphism), are also acquired resistance mechanisms (Martinez, 2014).

The targets of antibiotics differ according to the cell structure. Therefore, resistance mechanisms of gram-negative and gram-positive bacteria also differ (Marsik and Kumar, 2019). A quick overview of antimicrobial targets and the mechanism of resistance can be seen in Figure 2.

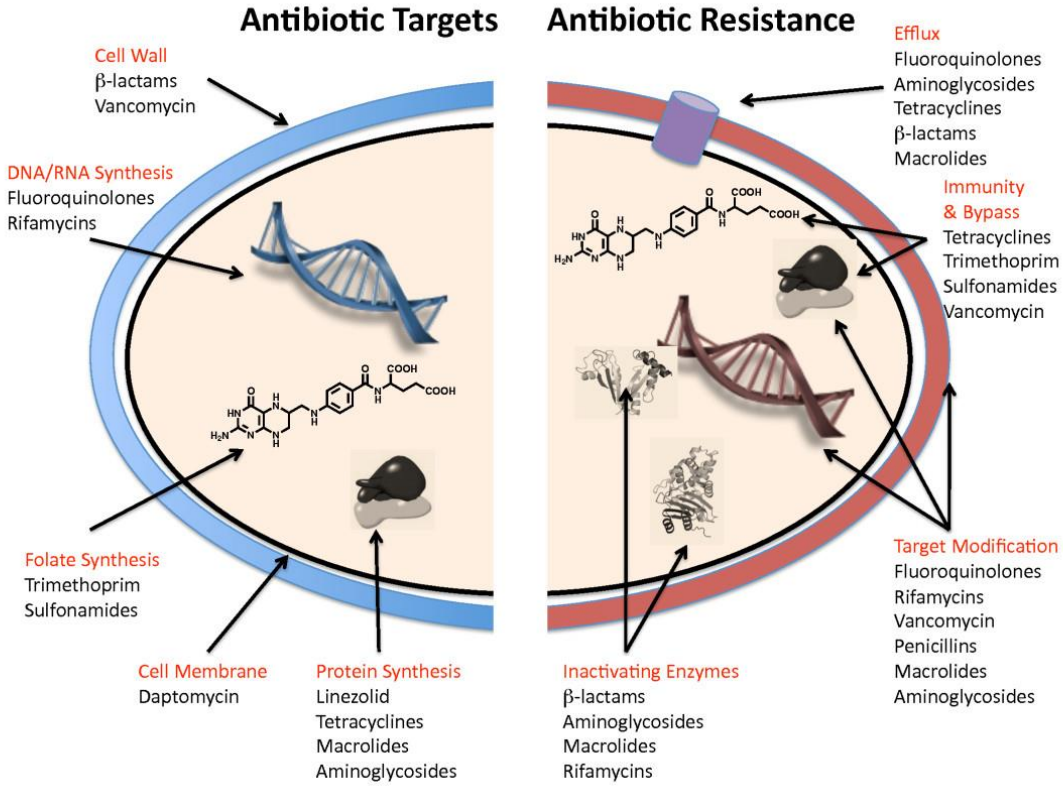


Figure 2. Antibiotic targets and mechanisms of resistance.
The picture shows mechanisms the targets of antibiotics and the antimicrobial resistance mechanisms. Source: Wright, 2010 (Creative Commons license).

Antimicrobial resistance is a long-term process mediated by DNA mutations. However, the presence of genetic mobile elements speeds up this process. When a subset of chromosomally encoded genes is recruited by gene-transfer elements and introduced in a new microorganism, this last one acquires the phenotypic characteristics developed by the original bacteria (Martinez, 2014). Besides, the selective pressure caused by the intensive use of antibiotics in human activities, plays an important role in the selection of resistant bacteria over wild type microorganisms.

Acquired genetic determinants related to the major resistance mechanism are linked to mobile elements such as plasmids, transposons, gene cassettes, integrative, and conjugative elements, etc (Schwarz, Loeffler and Kadlec, 2017). For this reason, the research of acquired genetic elements of resistance is essential to understand the epidemiology of bacteria and the search for new targets in antimicrobial development. An overview of the main antimicrobial resistance mechanism can be seen in the table below (Table 1).

Table 1. Main antimicrobial resistance mechanisms.

Antibiotic family	**Mechanism type**	**Specific Mechanism**	**Type of resistance**	**Acquired genetic determinants**	**Reference**
β-Lactams and Cephalosporins	Drug Uptake Limitation	Decreased numbers of porins, changed selective of porin, no outer cell wall	Natural	-	(Martínez-Martínez, 2008; Rossolini, Arena and Giani, 2017)
	Drug Target Modification	Alterations in penicillin-binding proteins (Gram pos)	Acquired	*mecA* gene	(Reygaert, 2009; Beceiro, Tomás and Bou, 2013)
	Drug Inactivation	β-lactamases (Gram pos, gram neg)	- Natural - Acquired	- -Genes from TEM, CMY, SHV, CTX-M, KPC, and other groups.	(Sanders, 1989; Bradford, 2001; Tooke *et al.*, 2019)
	Efflux Pumps	SMR efflux pumps, RND efflux pumps.	- Natural -Acquired	- -*tmexCD1-toprJ1* gene	(Kumar and Schweizer, 2005; Bay, Rommens and Turner, 2008; Du *et al.*, 2014; Lv *et al.*, 2020)
Carbapenems	Drug Uptake Limitation	Changed selectivity of porin	Natural	-	(Chow and Shlaes, 1991; Cornaglia *et al.*, 1996)
	Drug Inactivation	Carbapenemases (A, B and D β-lactamases)	- Natural -Acquired	- -KPC (*KPC*-2 to *KPC*-13), IMI (*IMI*-1 to *IMI*-3), and GES (*GES*-1 to *GES*-20) genes.	(Codjoe and Donkor, 2017)
Glycopeptides	Drug Uptake Limitation	Thickened cell wall, no outer cell wall	Natural	-	(Lambert, 2002; Bébéar and Pereyre, 2005)
	Drug Target Modification	Modified peptidoglycan	- Natural - Acquired	- -*van* gene	(Randall *et al.*, 2013) (Beceiro, Tomás and Bou, 2013; Cox and Wright, 2013)
Lipopeptides	Drug Target Modification	Modified net cell surface charge	Acquired	Mutations in genes (e.g. *mprF*)	(Stefani *et al.*, 2015)
Aminoglycosides	Drug Uptake Limitation	Cell wall polarity	Natural	-	(Miller, Munita and Arias, 2014)

Antibiotic family	Mechanism type	Specific Mechanism	Type of resistance	Acquired genetic determinants	Reference
	Drug Target Modification	Ribosomal mutation, and ribosomal subunit methylation	Acquired	Acquisition of *erm* genes	(Roberts, 2004)
	Drug Inactivation	Aminoglycoside modifying enzymes, acetylation, phosphorylation, adenylation	Acquired	*acc*, *aph*, and *ant* genes	(Ramirez and Tolmasky, 2010; Blair, Webber, *et al.*, 2015)
	Efflux Pumps	SMR efflux pumps, RND efflux pumps.	- Natural -Acquired	- -*tmexCD1*-*toprJ1* gene	(Kumar and Schweizer, 2005; Lv *et al.*, 2020)
Tetracyclines	Drug Uptake Limitation	Decreased numbers of porins	Natural	-	(Martínez-Martínez, 2008; Rossolini, Arena and Giani, 2017)
	Drug Target Modification	Ribosomal protection	Natural	-	(Spahn *et al.*, 2001; Roberts, 2003)
	Drug Inactivation	Antibiotic modification, oxidation	Natural	-	(Blair, Webber, *et al.*, 2015)
	Efflux Pumps	MFS efflux pumps. RND efflux pumps.	- Natural -Acquired	- *tmexCD1*-*toprJ1* gene	(Kumar and Schweizer, 2005; Du *et al.*, 2014; Lv *et al.*, 2020)
Chloramphenicol	Drug Target Modification	Ribosomal methylation	Acquired	Acquisition of *cfr* gene	(Kehrenberg *et al.*, 2005)
	Drug Inactivation	Acetylation of drug	Acquired	*cat* genes	(Schwarz *et al.*, 2004; Blair, Webber, *et al.*, 2015)
	Efflux Pumps	MFS efflux pumps, RND efflux pumps.	Natural	-	(Kumar and Schweizer, 2005; Du *et al.*, 2014)
Lincosamides	Drug Target Modification	Ribosomal methylation (Gram pos)	Acquired	Acquisition of *erm* genes	(Weisblum, 1995; Zhong *et al.*, 1999)
	Efflux Pumps	ABC efflux pumps, RND efflux pumps.	Natural	-	(Kumar and Schweizer, 2005; Lubelski, Konings and Driessen, 2007)
Macrolides	Drug Target Modification	Ribosomal mutation, methylation	Acquired	Acquisition of *erm* genes	(Zhong *et al.*, 1999; Roberts, 2004)
	Efflux Pumps	ABC efflux pumps, MFS efflux pumps, RND efflux pumps.	Natural	-	(Kumar and Schweizer, 2005; Lubelski, Konings and Driessen, 2007; Du *et al.*, 2014)
Oxazolidinones	Drug Target Modification	Ribosomal methylation	Acquired	Acquisition of *erm* genes	(Roberts, 2004)
	Efflux Pumps	RND efflux pumps.	Natural	-	(Kumar and Schweizer, 2005)

Antibiotic family	Mechanism type	Specific Mechanism	Type of resistance	Acquired genetic determinants	Reference
Fosfomycin	Drug Uptake Limitation	Defects or reduced expression of transporters (GlpT and UhpT)	Natural	-	(Falagas *et al.*, 2019; Liu *et al.*, 2020)
	Drug Target Modification	Overexpression of *MurA* gene.	Adquired	MurA mutations	(Falagas *et al.*, 2019; Liu *et al.*, 2020)
	Drug Inactivation	Fosfomycin inactivation by exerting glutathione-S-transferase	Adquired	Acquisition of *fos* genes (*fosA, fosA2, fosA3, fosB, fosC* and *fosX*).	(Falagas *et al.*, 2019; Liu *et al.*, 2020)
Streptogramins	Efflux Pumps	ABC efflux pumps	Natural	-	(Kumar and Schweizer, 2005; Lubelski, Konings and Driessen, 2007)
Fluoroquinolones	Drug Target Modification	DNA gyrase modification (Gram neg). Topoisomerase IV modification (Gram pos). DNA gyrase protection from quinolone inhibition.	Acquired	-Mutation in genes like *parC*, *gyrA*, *grlA*, etc. -Acquisition of *qnr* genes	(Hawkey, 2003; Redgrave *et al.*, 2014; Hooper and Jacoby, 2015)
	Drug Inactivation	Acetylation of drug	Acquired	*AAC(6¢)-Ib* genes	(Robicsek *et al.*, 2006; Blair, Webber, *et al.*, 2015; Hooper and Jacoby, 2015)
	Efflux Pumps	MATE efflux pumps, MFS efflux pumps, RND efflux pumps.	- Natural -Acquired	- *-tmexCD1-toprJ1* gene	(Kumar and Schweizer, 2005; Kuroda and Tsuchiya, 2009; Du *et al.*, 2014; Hooper and Jacoby, 2015; Lv *et al.*, 2020)
Sulfonamides	Drug Target Modification	DHPS reduced binding, overproduction of resistant DHPS	Acquired	Mutations in *folP* and *dhps* genes	(Huovinen *et al.*, 1995; Vedantam *et al.*, 1998)
	Efflux Pumps	RND efflux pumps.	Natural	-	(Köhler *et al.*, 1996; Kumar and Schweizer, 2005)
Trimethoprim	Drug Target Modification	DHFR reduced binding, overproduction of DHFR	Acquired	Location of trans poson Tn7, acquisition of DHFR genes (*dfrA*), and mutation DHFR genes (*dfrB*).	(Huovinen *et al.*, 1995; Rossolini, Arena and Giani, 2017)

Antibiotic family	Mechanism type	Specific Mechanism	Type of resistance	Acquired genetic determinants	Reference
	Efflux Pumps	RND efflux pumps.	Natural	-	(Köhler *et al.*, 1996; Kumar and Schweizer, 2005)
Polymyxin	Drug Uptake Limitation	Capsule Shielding	Natural	-	(Aghapour *et al.*, 2019; Moffatt, Harper and Boyce, 2019)
	Drug Target Modification	-Modification lipopolysaccharide structure (LPS), addition of 4-amino-L-arabinose (L-Ara4N), phosphoethanolamine (PEtn), or galactosamine. -Loss of LPS .	-Natural - Acquired	- - PEtn transferase genes (*mcr*-1 to *mcr*-9). -Mutations in genes *lpxA*, *lpxC* or *lpxD*.	(Aghapour *et al.*, 2019; Moffatt, Harper and Boyce, 2019)
	Drug Inactivation	Production of colistin and a putative serine protease	Natural	-	(Ito-Kagawa and Koyama, 1980; Aghapour *et al.*, 2019; Moffatt, Harper and Boyce, 2019)
	Efflux Pumps	Tripartite efflux system MtrCDE	Natural	-	(Tzeng and Stephens, 2015; Aghapour *et al.*, 2019; Moffatt, Harper and Boyce, 2019)

ABC: ATP-Binding Cassette family, MATE: Multidrug and Toxic compound Extrusion family, SMR: Small Multidrug Resistance family, MFS: Major Facilitator Superfamily, RND: Resistance-Nodulation-cell Division superfamily. Adapted and modified from Reygaert, (2018): An overview of the antimicrobial resistance mechanisms of bacteria.

2.2.1. World concern on antimicrobial resistance.

The overuse of antibiotics in humans and animals has produced the selection of multi resistant bacteria (resistant to more than 2 antimicrobial groups)(Magiorakos *et al.*, 2012). However, MDR *Salmonella* is defined by its resistance to all first-line antimicrobials (ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol)(Wain and Kidgell, 2004).

The increasing rate of antimicrobial resistance has become the main public health problem in the last century. This problematic situation has increased the levels of morbidity and mortality in humans and animals. Therefore, costs of health care attention and husbandry have also been increased (WHO, 2017). Therefore, WHO adopted the Global Action Plan on Antimicrobial Resistance in 2015 (WHO, 2015a). This project has the goal to ensure the successful continuity of current treatments and combat the health threat posed by increasing antimicrobial resistance.

The global action plan on antimicrobial resistance recognizes the need of a multisectoral action framed in a One Health context. This plan emphasizes the need of a general surveillance program, that include health care services, the environment, and the food chain. However, few countries have reached this objective, principally due to ineffective public health surveillance

systems, lack of expertise, insufficient laboratory capacity, and poor data management (WHO, 2015c).

Retail food (animal-derived food products) as well as food-producing animals (sick and healthy) are the main source of resistance bacteria for humans. Therefore, the integration of these sectors is fundamental in any antimicrobial surveillance program. In this context, antimicrobial surveillance should focus in production animals, since these samples can give an unbiased measure of antimicrobial resistance in the beginning of the food chain (WHO, 2017).

2.3. Methods for the study of *Salmonella*.

The study of *Salmonella* integrates different techniques, from isolation up to molecular characterization. These microbiological methods are internationally accepted and must be normalized in laboratories according their capacities for *Salmonella* reporting (WHO, 2017). An overview of commonly available techniques for the study of *Salmonella* is described below.

2.3.1. Bacterial culture and isolate identification.

The development of new technologies has allowed to have an in-depth knowledge about genetic factors of *Salmonella*. However, primary identification and isolation of these bacteria is done with traditional microbiological techniques (Andrews *et al.*, 2021).

Salmonella, unlike other enterobacteria, is usually in low concentrations. However, low concentrations of this pathogen can colonize susceptible hosts (Cosby *et al.*, 2015). Therefore, the presence/absence tests are more important than the quantification of *Salmonella*.

The isolation process of *Salmonella* starts with a non-selective pre-enrichment. This stage aims to stabilize *Salmonella* cells in the sample, rise their numbers and improve the probability of their detection (Gonzalez Pedraza *et al.*, 2014; Andrews *et al.*, 2021).

Next, a selective enrichment is performed. This stage inhibits accompanying flora growth and enhances the development of low concentrations of *Salmonella* in the sample. Besides, a prior selection of not-typhoidal serotypes (mobile *Salmonella*) can be carried out using selective-differential media. Differentiation of *Salmonella* colonies on the media used for selective isolation is performed according to their phenotype (ISO, 2014; Andrews *et al.*, 2021). The main culture media used for selective and non-selective culture are shown in the table 2.

Table 2. Culture media used for the isolation of *Salmonella*.

Pre-enrichment	Selective enrichment	Selective-differential media
• Lactose Broth (LB). • Tryptic Soy Broth (TSB). • Nutritive Broth (NB). • Buffered Peptone Water (BPW).	• Rappaport-Vassiliadis Broth • (RV). • Rappaport-Vassiliadis Soja Broth (RVS). • Müller-Kauffmann Tetrathionate-Novobiocine Broth (MKTTn). • Tetrathionate Broth (TTB). • Selenite Cystine Broth (SCB).	• Hektoen Enteric (HE) Agar. • Bismuth Sulfite (BS) Agar. • Xylose Lysine Deoxycholate (XLD) Agar. • Deoxycholate Citrate Agar (DCA). • Harlequin *Salmonella* ABC Medium. • Deoxycholate Citrate Lactose Sucrose (DCLS) Agar. • Rambach agar (RA).

	• Selenite-F Broth (CFB). • Modified Rappaport Vassiliadis Semi-Solid Agar (MSRV).	• SMID (*Salmonella* Detection and Identification) Chromogenic Agar. • Xylose Lysine Tergitol 4 (XLT4) Agar. • *Salmonella* Shigella (SS) Agar. • McConkey agar (MAC). • Mannitol Lysine Crystal Violet Brilliant Green (MLCB) Agar. • Brilliant Green Agar (BGA).

Fount: (Soria, 2012)

Once suspicious colonies have developed, a set of biochemical tests are necessary to confirm the presence of *Salmonella*. The culture medium used for these tests include Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA), Urea Broth, and Medium Sulfide Indole Motility (SIM)(Jorgensen *et al.*, 2015; Andrews *et al.*, 2021). An overview of biochemical tests used to identify *S. enterica* is shown below (Table 3).

Table 3. Biochemical test for *S. enterica* identification.

Biochemical tests	Expected result
Lactose	-
O-nitrophenyl-beta-D-galactopyranoside (ONPG)	-
H_2S production	+
Glucose (fermentation)	+/ with gas
Dulcita (fermentation)	+
Adonite (fermentation)	-
Lysine decarboxylase	+
Ornithine decarboxylase	+
Arginine dihydrolase	+
Urea (hydrolysis)	-
Indole	-
Motility	According to the serotype
Methyl red	+
Voges Proskauer	-
Simmons citrate	+
Malonato	-

Fount: (Caffer and Terragno, 2001)

2.3.2. Antimicrobial susceptibility testing.

Recognizing the susceptibility or resistance of *Salmonella* is an important task after its isolation. The objective of these tests is to identify the best treatment option in an infection process. The most commonly used methods provide either quantitative or qualitative results and include broth microdilution and diffusion methods (Jorgensen and Ferraro, 2009).

A) **Broth dilution test.** This quantitative technique allows to determine the minimal inhibitory concentration (MIC). For this technique, it is necessary to prepare sequential dilutions of antibiotics (eg, 1, 2, 4, 8, and 16 mg/mL) in a liquid growth medium. These

dilutions will be dispensed in either test tubes or microplates. Next, these dilutions will be inoculated with a standardized bacterial suspension and overnight incubated. Finally, the lowest concentration that does not allow bacterial growth (evidenced by turbidity) will be considered as the MIC. For its precision, this technique is considered the gold for the identification of antimicrobial resistance (Jorgensen and Ferraro, 2009; CLSI, 2019).

B) **Disk diffusion test.** This qualitative technique allows to evaluate the effectiveness of an antibiotic by classifying it as susceptible, intermediate, or resistant. To perform this test is necessary to apply a standardized bacterial suspension to the surface of a Mueller-Hinton agar plate. Next, commercially prepared paper antibiotic disks are placed on the inoculated agar surface. Finally, after overnight incubation, the growth inhibition zones around each disk are measured. The diameter of inhibition around each antibiotic must be interpreted according to the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST)(CLSI, 2019; EUCAST, 2021).

C) **Automated instrument systems.** Antimicrobial susceptibility test can also be performed by automatic equipment. These systems allow easy management and shorter periods than manual procedures. The instruments approved by de FDA for use in the USA included MicroScan WalkAway (Siemens Healthcare Diagnostics), BD Phoenix Automated Microbiology System (BD Diagnostics), Vitek 2 System (bioMe´rieux), and Sensititre ARIS 2X (Trek Diagnostic Systems)(Jorgensen and Ferraro, 2009).

2.3.3. *Salmonella* serotyping

Serotyping is a technique that allows *Salmonella* classification according to its antigenic structure, surpassing the sub-specie level (Sanderson and Nair, 2013). This characterization is important in epidemiological studies because it gives an extra degree of resolution. Serotyping allows to have clues about the source and transmission ways of *Salmonella* in an outbreak (Herikstad, Motarjemi and Tauxe, 2002).

This technique involves the identification of somatic surface antigens (LPS, O antigens) and flagellar antigens (proteins, H antigens) using antigen-antibody reactions. For this, Kauffmann-White scheme is used as the reference (Barrow and Methner, 2013).

Kauffmann-White scheme uses a formula divide in four parts to name the *Salmonella* serotypes (Issenhuth-Jeanjean *et al.*, 2014). For example, the monophasic variety of *Salmonella* Typhimurium is written as follows:

Salmonella Typhimurium 4,5,12: i:-

(a) Serotype: Typhimurium
(b) Somatic antigens (O or LPS): 4,5,12
(c) First flagellar phase(H1): i
(d) Second flagellar phase (H2): - (it is no present).

Currently, the traditional serotyping process is being replaced by genetic markers recognized either by PCR or Whole Genome Sequencing (WGS). These techniques allow a most accurate serotype identification, limiting human errors of interpretation (Banerji *et al.*, 2020; Uelze *et al.*, 2020).

2.3.4. Molecular diagnostic of *Salmonella*.

Although microbiological diagnostic of *Salmonella* is an accurate technique, the time necessary to obtain a valid result can reach some days. This aspect can be a problem in situations like outbreaks and clinical cases that require fast response. In these cases, molecular diagnostic tools are convenient options to consider.

The Polymerase Reaction Chain (PCR) is a fast technique that allows an enzymatic in-vitro amplification of specific DNA fragments. The natural ability of DNA polymerases to create identical copies of DNA using thermal cycles is used to obtain a high number of DNA fragments. These DNA fragments are detected through electrophoresis or fluorescence linked to probes (Sandoval, Meza and Floresvillar, 2016). This technique has been used for the detection of several pathogens, including *Salmonella* (Y. Liu *et al.*, 2019). The PCR technique is not limited to pathogen diagnostics but also for virulence and resistance genes detection (Adesiji, Deekshit and Karunasagar, 2014). Besides, modifications of this technique are used for bacteria fingerprint and serotype characterization (Jean-Gilles Beaubrun *et al.*, 2012; Hashemi and Baghbani-Arani, 2015).

The *invA* gene arbored in the island of pathogenicity 1 (SPI1) is related to the invasion to epithelial cells of the gut (Andesfha *et al.*, 2019). This gene has a specific sequence for the *Salmonella* genus, being commonly used as a target for *Salmonella* diagnostic through PCR (Rahn *et al.*, 1992; Bai *et al.*, 2018). Since PCR designated for *invA* gene detection has been demonstrated to be fast, specific, and highly sensible, the use of this target gene has become the international standard for molecular *Salmonella* detection (Malorny *et al.*, 2003).

2.3.5. Genomic fingerprinting of *Salmonella*.

Although *Salmonella* serotyping gives clues about the epidemiology of these bacteria, the discriminatory power of this technic as an epidemiological tool by itself is limited (Albufera et al., 2009). However, since each serotype can be divided into several genotypes, the use of fingerprint techniques can give further and accurate data (Hashemi and Baghbani-Arani, 2015). The main popular genomic fingerprinting techniques are described below.

a) Pulsed-field gel electrophoresis (PFGE). This technique has been the gold standard for molecular typing of *Salmonella* for many years. PFGE has been used to track outbreaks of bacterial diseases, to study their evolution, or to research their dynamic in husbandry systems (Dauphin, Ragimbeau and Malle, 2001; Parizad, Parizad and Valizadeh, 2016; Vinueza-Burgos *et al.*, 2019).

The segmentation of the total bacterial DNA is the base of the PFGE technique. For this, a restriction enzyme is used (Xbal in the case of *Salmonella*). These fragments are then separated by electrophoresis, whose electric field changes direction sequentially. In this way, the separation of fragments over 20 kb is improved, and specific bands for each strain are obtained (Sharma-Kuinkel, Rude and Fowler, 2016).

However, since this is a time-consuming and labor-intensive technique, its popularization in different laboratories has been difficult. Besides, the power of discrimination of this technique differs considerably among serotypes (Winokur, 2003).

b) Random amplification of polymorphic DNA - PCR (RAPD-PCR). This technique, based on PCR, uses unspecific primers to randomly amplify different regions in the bacterial genome. Next, the pattern of products generated in this process and visualized through electrophoresis is used as a genetic fingerprint (Wolfe and Liston, 1998). In this technique one sequence is used as forward and revers primers. The bands generated in this PCR are denominated locus. The presence or absence of locus between individuals is produced by changes or lacks in sites of primer hybridization. Besides, the size of the amplified locus can be modified by the insertion or deletion of nucleotides in the sequence between the hybridization sites (Williams *et al.*, 1990).

The easy implementation of RAPD-PCR in a broad range of laboratory settings has allowed its use in diverse genomic studies applied on several species. However, its limited reproducibility has made this technique disused (Hashemi and Baghbani-Arani, 2015).

c) Repetitive extragenic palindromic - PCR (REP-PCR). The REP-PCR is a fingerprint technique based in the amplification of conserved repetitive sequences dispersed in the bacteria genome (Mohapatra, Broersma and Mazumder, 2007). These sequences are classified in four types: Sequences BOX, Repetitive extragenic palindromic (REP), Enterobacterial repetitive intergenic consensus (ERIC), and Polytrinucleotide $(GTG)_5$ sequences (Versalovic et al., 1994). The discriminatory power of these techiques is variable, however studies carried out by Mohapatra, Broersma and Mazumder, (2007) placed the$(GTG)_5$-PCR as the most suitable method betwent the REP variants for molecular typing.

In these techniques, diverse regions of DNA flanked by rep sequences are amplified by PCR producing specific patterns for each isolate. These techniques unlike PFGE are characterized by its simplicity since they do not require restriction enzymes or especial electrophoresis technique. Besides, they are fast and with and relative low cost (Olive and Bean, 1999; Martín-Lozano *et al.*, 2002). However, the discrimination power of REP techniques remains lower than PFGE.

d) Multilocus sequence typing (MLST). The introduction of PCR and the improvement of the sequencing techniques implemented by Sanger technology opened new possibilities in bacteria typing. Of these new technologies, the most successful has undoubtedly been Multilocus sequence typing (MLST)(Uelze *et al.*, 2020).

This technique consists of the PCR amplification and sequencing of 7 housekeeping genes. These sequences will be used to assign a profile to each analyzed bacterium. The information generated by this technique can be easily shared between laboratories and used to compare bacteria isolates from all over the world (Ibarz Pavón and Maiden, 2009).

The MLST technique detects changes in the DNA that are not phenotypically perceptible. However, the discriminatory power of this technique is only slightly better than serotyping. Therefore, it is not recommended for the study of highly related strains (Achtman *et al.*, 2012).

With the development of new sequencing techniques and the popularization of WGS, new variations of MLST will be developed to balance the deficiencies of this technique. The Core genome MLST (cgMLST) and Whole-genome MLST (wgMLST) are MLST types that use the same principle but include hundreds or thousands of additional genes for the analysis. However, as there is no official central body to centralize, curate, and normalize existing schemes and those that appear every day, investigations cannot be compared. Furthermore, the pipelines used

by different services (e.g., Enterobase and Ridom SeqShere +) produce several allele differences, generating non-standardized data (Uelze *et al.*, 2020).

e) WGS and Single nucleotide polymorphism (SNP) calling. The last approach in bacteria typing is the differentiation of SNP among strains. These differences are identified by mapping the investigated genomes against a highly related reference. This type of analysis considers only those sequences covered by the reference genome, creating a core reference set. Each SNP located matching the draft and this reference set becomes part of a matrix of SNP distances that can then be interpreted in phylogenetic analyzes such as neighbor-joining and maximum likelihood phylogenetic trees (Felsenstein, 1981; Bakker *et al.*, 2011).

For this technique, it is recommended to work with a reference closed genome that is as highly related as possible to the investigated one. It must be noted that the use of a draft genome as reference substantially decreases the precision of the technique. Moreover, since the SNP calling is performed from a coverage-based set, the use of a low related genome (or a draft genome) would decrease the possibility of finding SNPs (Uelze *et al.*, 2020).

Besides, in order to perform this technique is necessary to select suitable algorithms to call, assure the quality, and filter the SNPs. The most popular tools and pipelines used for this analysis are SAMtool (Li, 2011), Freebayers (Pegueroles et al., 2020), Snippy (Seemann, 2015), GATK (DePristo et al., 2011), CFSAN (Davis et al., 2015), DMSTree (Zhou et al., 2020), etc.

The capacity of SNP analysis for the discrimination of strains is congruent to cgMLST and wgMLST. However, cgMLST and wgMLST detect mutations in specific alleles, making their discrimination power less accurate (Uelze et al., 2020). Additionally, SNP analysis can be used to perform evolutionary models for phylogenetic inference and has been used to track and analyze several *Salmonella* outbreaks worldwide (Bakker et al., 2011; Taylor et al., 2015; Inns et al., 2017; Pearce et al., 2018).

2.4. Whole genome sequencing.

Sanger sequencing has been the gold standard for identifying genetic variants in the last few years. However, the limitations of this technique regarding the size and number of amplified fragments as well as its cost have impulse the development of new technology (Shendure and Ji, 2008; Tucker, Marra and Friedman, 2009). In this context, the technological advances performed since 2007 has allowed the development of new and improved techniques, known as next-generation sequencing (NGS). The NGS, also named massive parallel sequencing (MPS), allowed the number of bases sequenced per price unit to grow exponentially (Stein, 2010). Besides, these techniques have the potential to identify all kinds of genetic variation such as single nucleotide polymorphisms (SNPs), small insertions and deletions, and structural variants (inversions, translocations, deletions, and duplications) in the same assay (Rodríguez-Santiago and Armengol, 2012). This assay is named whole-genome sequencing (WGS).

Although the instruments used in NGS manage different technical approaches, the conceptual work scheme is similar for all of them (Mardis, 2008; Metzker, 2010; Liu *et al.*, 2012) Thus, the main stages in this process are:

a) Library preparation: this stage includes the DNA fragmentation and the ligation of adapter sequences to the ends.

b) Amplification and clustering: here, the DNA fragments are clonally amplified and pooled together as entities to be sequenced.
c) Sequencing and Imaging: the sequence is read using either alternating cycles of cyclic reversible termination (CRT) and “imaging” (e.g., Illumina technology), or trough out pH measurements when nucleotides are read in the sample sequence (Ion Torrent).

Each CTR reaction uses reversible terminator to incorporate fluorescent labeled nucleotides in the sequencing process. These labeled nucleotides are then "photographed" during imaging and further processed. These short sequences are named “reads” (Morozova and Marra, 2008).

On the other hand, the pH measurements approach, use a sensor named ISFET (Ion-Sensitive Field-Effect Transistor). This sensor measures the pH change produced when a nucleotide is ligated to the strand and a hydrogen ion is released. The ISFET sensor measure the complementary of bases. Thus, different nucleotides are sequentially faced to the strand to sequenced up to the complementary base is detected (Pennisi, 2010).

Two concepts must be considered to understand the process and generate reliable results from NGS techniques, “coverage” and “depth”. The coverage or breadth of coverage is the percentage of sequenced bases aligned with a reference genome. On the other hand, the depth or depth of coverage represent the average number of times that each base in the genome is sequenced (Sims *et al.*, 2014).

Other important aspects to consider in the NGS is the power of computational analysis and bioinformatic knowledge. These sequencing technologies produce an unprecedented amount of data that a common computer cannot handle (Stein, 2010). Furthermore, to perform an analysis of this experimental data with biological sense is necessary to incorporate all the existing relevant information. Therefore, computer science skills are absolutely necessary.

Although there are several platforms and technologies to perform WGS, the two main ones belong to Illumina and Thermo Fisher (Ion Torrent). However, Illumina sequencing is the main one used on WGS. So, most of WGS information in data contained in databases is obtained by Illumina technology (Seth-Smith *et al.*, 2019) since it’s the cheapest one and give a good quality of results (Lahens *et al.*, 2017; Preston, VanZeeland and Peiffer, 2021).

2.5. Ecuador and its poultry meat production.

Ecuador is a South American country crossed by the Andes Mountains and has access to the Pacific Ocean. These particularities give the country four natural regions: Amazon Region, Andes Region, Coastal Region, and Galapagos Islands (Figure 3).

Figure 3. Natural regions of Ecuador.
Source: Google Earth Pro. In the graphic, it is possible to appreciate the Andes Mountain dividing Ecuadorian territory from north to south. (1) Amazon Region, (2) Andes Region, (3) Coastal Region, (4) Galapagos Islands.

Currently, Ecuador surpasses 17 million inhabitants. This population is distributed into 24 provinces, being Pichincha and Guayas the most inhabited with 35.07% of the population (INEC, 2021). One of the main sources of animal protein for Ecuador's population is poultry production. Ecuador produces around 263 million broilers annually. These birds are exclusively consumed in the local market. The estimate per capita consumption of chicken meat was 28.3 Kg/year in 2020 (CONAVE, 2021). However, this amount is low compared to other countries in the region such as Peru and Brazil with 50.3 and 45.3 kg/year respectively (DGPA, 2019; ABPA, 2020). Turkey constitutes the second source of avian protein, however, its housing capacity is limited to 500,000 birds (CGSIN and MAGAP, 2015).

Integrated poultry companies produce around 90% of Ecuadorian poultry. These systems generally have industrialized processes and include segments such as breeders, hatcheries, feed plants, broiler farms, slaughterhouses, and sometimes they own markets and restaurants. However, a considerable proportion of live birds are commercialized to middlemen (Vinueza, 2017).

Even though industrialized slaughterhouses in Ecuador are regulated by sanitary entities (INEN, 2012; AGROCALIDAD and MAGAP, 2013), just 35% of national poultry production is slaughtered in these installations. Besides, several processes in industrialized slaughterhouses are manually performed or lack monitorization systems resulting in specific hotspots of microbiological contamination (Vinueza, 2017).

Whether the birds are slaughtered in regulated slaughterhouses or not, the role of integrated poultry companies is critical for the supplying of chicken meat. Therefore, understanding and controlling pathogens in the different stages of these production systems is a key factor to limit the transmission of foodborne diseases.

The objectives of this study were:

a) To investigate the epidemiology and population dynamics of *Salmonella* serotypes in two poultry integrations using a whole-genome sequencing approach.
b) To investigate the presence of genetic determinants of antimicrobial resistance (GDAMR) in these strains that can potentially express phenotypic resistance.
c) To describe plasmids that can harbored GDAMR.

3. Material and methods

3.1. Study design

For this study, *Salmonella* isolates originating from two integrated poultry companies (integrations A and B) were characterized and compared using WGS. *Salmonella* was isolated from the feed mill plants, broiler farms, and slaughterhouses comprising both integrations. Specific details on sampling design and *Salmonella* isolation have been published in previous papers (Villagómez, Logacho and Vinueza, 2017; Vinueza-Burgos *et al.*, 2019). Briefly, several samples were taken in each stage throughout the productive chain in both integrations according to the size and requirements of each company. The sampling in each location was carried out in aseptic conditions. Next, the samples were transported to the laboratory in refrigeration and processed according to the ISO:6579 (ISO, 2014). The *Salmonella* isolates were stored at -80 °C in Trypticase soy broth (TSB) with glycerol in a proportion of 3:1 up to the extraction of their DNA for WGS. The samples characteristics along the productive chain as well as the distribution of *Salmonella* isolates within each integrated poultry company are presented in Table 4.

Table 4. Distribution of *Salmonella* isolates used in this study within each integrated poultry company.

Sample origin	Sampling location	Sample taken	Integration A	Integration B	Total
Raw feed materials	Feed mill plant	25g of each ingredient	1	30	31
Compound feed	Feed mill plant	25g of each type of feed	NA	2	2
Transport paper*	Broiler farms	25g of transport paper with meconium	1	1	2
Overshoes**	Broiler farms	25g of boot swabs after walking along the poultry shed 3 times.	28	44	72
Caeca content***	Slaughterhouse	25g of cecal content	2	3	5
Skin after final washing	Slaughterhouse	25g of breast skin	22	50	72
Carcasses (Skin after chilling)	Slaughterhouse	25g of breast skin	14	44	58
Turkey house	Turkey farm	25g of boot swats after walking along the poultry shed 3 times.	NA	1	1
Total			68	175	243

*One day old chicks
** Broiler-litter sweep swab of 30 days-old chicken.
*** Samples were taken on the day of slaughter.
NA: no isolate was recovered from these locations for WGS.

3.2. Methods

3.2.1. DNA extraction and whole-genome sequencing

All *Salmonella* isolates were confirmed by PCR as previously described (Vinueza-Burgos *et al.*, 2019). Genomic DNA was then extracted and purified using Wizard® Genomic DNA

Purification Kit (Promega, MD), and the concentrations measured using a Qubit® fluorometer using the Qubit dsDNA High Sensitivity (HS) Assay kit (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA). Additionally, quality assessments were made using the NanoDrop 2000 UV-Vis (Thermo Fisher Scientific, Waltham, MA, USA) for determination of A260/280 values. DNA extracts containing a minimum concentration of 10 ng/µl and an A260/280 ratio of 1.75-2.05 were used for posterior analysis. DNA extracts outside this range that were sequenced and found to provide acceptable data quality based on coverage (>40X) and other quality assessments inherent in the analysis pipelines below were included in the analysis.

WGS was performed using the MiSeq platform (Illumina, San Diego, USA) according to the harmonized FDA GenomeTrakr/CDC PulseNet protocol (CDC PulseNet, 2018). The Trimming, assembly and quality control of raw reads was performed using the EnteroBase pipeline (Zhou *et al.*, 2020).

3.2.2. Bioinformatics analyses

Raw reads were submitted to EnteroBase (Zhou *et al.*, 2020) in order to perform the primary analysis and confirm serotype designation using the SISTR algorithm. EnteroBase tools were also used to determine the MLST Sequence Type (ST) profile of each isolate (Acthman scheme) and perform SNP tree analysis of the isolates in each collection. Visualization of the SNP tree analysis and related metadata was performed using IToll (Letunic and Bork, 2019). Additionally, a Principal Components Analysis (PCA) was implemented in the adegenet package on RStudio (Jombart, 2008; Jombart and Ahmed, 2011) using polymorphic sites identified by the Enterobase in the SNP tree construction. This assay was performed to investigate the population structure and the genetic relationship between individuals from different sampling locations.

The identification of genes that determine antimicrobial resistance were performed using the ResFinder database ((Zankari *et al.*, 2012; Bortolaia *et al.*, 2020). Virulence genes were identified using the Virulence Factor database (B. Liu *et al.*, 2019) and genes that confer resistance to disinfectants and heavy metals were accessed using the MEGARes database (Doster *et al.*, 2019). All databases used were updated to 9/12/2020, and all analyses were performed using a mass screening of contigs in ABRicate software (Seemann, 2020).

To identify functional nitrofurantoin resistance-associated mutations, the wild type sequences of *nfs*A and *nfs*B genes (oxygen insensitive nitro reductase enzymes) were copied from the chromosome of *S. enterica* accession number NC_003197. All genomes were then analyzed using the map to reference tool in the Geneious prime (V. 2021.0.3) software. Sequences that possessed mutations were *in silico* translated and aligned with the wild type proteins sequences to visualize amino acid residue mutations.

Plasmid finder database (Camacho *et al.*, 2009; Carattoli *et al.*, 2014) with ABRicate software were used to identify the plasmid incompatibility groups. In order to characterize the pESI-like megaplasmids, sequences were mapped to the p-F219 plasmid (GeneBank accession number CP038508) described in *S.* Infantis that were isolated from broiler production systems in Peru (Vallejos-Sánchez *et al.*, 2019) using the map to reference tool in the Geneious Prime software. Consensus sequences identified in each genome were depicted using Blast Atlas (Buckingham and Hogan, 2010).

4. Results

The present study included the DNA of 243 *Salmonella* isolates. The DNA concentration of all samples ranged from 28 to 104 ng/μL and the A260/280 ratio between 1.78-2.06. The resulting sequences integrated into the study exhibited a depth of coverage between 50 and 419X (average of 168.8X) and an N50 between 23,137 and 351,007 (average of 98,683). Other assembly stats are descripted in the Annex 2.

4.1. Serotype identification

Twelve different serotypes were identified among the studied isolates, three serotypes from integration A, and 11 from integration B. *Salmonella* Infantis was the most prevalent serotype in both integrations, accounting for 82.3% (n=200) of all isolates. Interestingly, *S.* Infantis and *S.* Amsterdam were present in both integrations while the remaining serotypes were isolated in only one of the two companies. Furthermore, *S.* Infantis was observed throughout the production chain (Table 5).

Table 5. Frequency and origin of *Salmonella* serotypes in each integration

Serovar	**integration A**		**Integration B**	
	%	Origin (n)	%	Origin (n)
Infantis	95.6	CC (2), O (26), SC (14), SW (22), TP (1)	77.1	CF (1), RM (4), O (37), CC (3), SC (40), SW (50)
Amsterdam	2.9	O (2)	10.3	RM (18)
Liverpool	-	-	5.1	RM (8) O (1),
Havana	-	-	2.3	O (1), SC (3)
Javiana	-	-	1.1	O (1), TP (1)
Saintpaul	-	-	1.1	O (1), SC (1)
Mbandaka	1.5	RM (1)	-	-
Soerenga	-	-	0.6	CF (1)
Albany	-	-	0.6	O (1)
Muenchen	-	-	0.6	O (1)
Uganda	-	-	0.6	O (1)
I 4,[5],12:i:-	-	-	0.6	T (1)
Total	**100**	**68**	**100**	**175**

Number of isolates (n), Raw feed materials (RM), Compound feed (CF), Transport paper (TP), Overshoes (O), Caeca content (CC), Skin before chilling (SW), Skin after chilling (SC); Turkey house (T)

4.2. Antimicrobial resistance

a) *Salmonella* Infantis

Most *S.* Infantis isolates possessed genetic determinants of antimicrobial resistance (GDAMR) against two or more antimicrobial classes (Tables 6 and 8). In fact, 82.5% of these isolates were grouped into patterns 1, 2, and 3, which possessed genetic determinants of resistance against nine, eight, and six antimicrobial classes, respectively. On the other hand, patterns 7 and 14 (integration B-specific) contained genetic determinants for two and three antimicrobial classes, respectively. Patterns 1, 2, 4, 8 and 10 were common to isolates originating from both integrations.

Table 6. Genome-derived antimicrobial resistant patterns in *S.* Infantis isolates.

Nº	**AMR Pattern**	**Nº of antimicrobial classes**	**No. of isolates (%)**		
			Integration A	**Integration B**	**Total**

1	ABPRFQSTN	9	46 (70.8)	51 (37.8)	97 (48.5)
2	ABPRQSTN	8	4 (6.2)	33 (24.4)	37 (18.5)
3	ABQSTN	6	-	31 (23)	31 (15.5)
4	ABRFQSTN	8	6 (9.2)	2 (1.5)	8 (4)
5	ABPQSTN	7	-	7 (5.2)	7 (3.5)
6	APRQSTN	7	-	4 (3)	4 (2)
7	AQ	2	-	4 (3)	4 (2)
8	ABPFQSTN	8	2 (3.1)	1 (0.7)	3 (1.5)
9	ABPRFQN	7	3 (4.6)	-	3 (1.5)
10	APRFQSTN	8	1 (1.5)	1 (0.7)	2 (1)
11	ABFQSTN	7	1 (1.5)	-	1 (0.5)
12	ABPRQN	6	1 (1.5)	-	1 (0.5)
13	ABRQSTN	7	1 (1.5)	-	1 (0.5)
14	AQN	3	-	1 (0.7)	1 (0.5)
Total of isolates			**(65)**	**(135)**	**(200)**

Aminoglycoside (A), Beta-lactam (B), Phenicol (P), Trimethoprim (R), Fosfomycin (F), Quinolone (Q), Sulfonamide (S), Tetracycline (T), Nitrofurans (N).

b) Other *Salmonella* serotypes

Isolate belonging to serotypes Havana, I 4,[5],12:i:-, Javiana, Muenchen, and Saintpaul also possessed Antimicrobial Resistance (AMR) genes against >2 antimicrobials classes. The most common combination of resistance genes that was observed among the isolates encoded against both aminoglycosides and quinolones (Table 7).

Table 7. Genome-derived antimicrobial resistant patterns and sequence types of non *S.* Infantis serovars isolated at Integrated poultry companies.

Serovar	ST	AMR Pattern	Nº of antimicrobial classes	No. of isolates (%)	
				Integration A	Integration B
Albany	292	AQ	2	-	1 (2.5)
Amsterdam	2090	AQ	2	2 (66.7)	18 (45)
Havana	588	ARQS	4	-	2 (5)
		ABPRMQST	8	-	1 (2.5)
		ARQST	5	-	1 (2.5)
I 4,[5],12:i:-	19	ABS	3	-	1 (2.5)
Javiana	1674	AQ	2	-	1 (2.5)
		ABQ	3	-	1 (2.5)
Liverpool	1959	AQ	2	-	9 (22.5)
Mbandaka	413	AQ	2	1 (33.3)	-
Muenchen	83	AQT	3	-	1 (2.5)
Saintpaul	50	A	1	-	1 (2.5)
		ABPRMST	7	-	1 (2.5)
Soerenga	1659	AQ	2	-	1 (2.5)
Uganda	684	AQ	2	-	1 (2.5)
Total of isolates				(3)	(40)

Sequence Type (ST), Aminoglycoside (A), Beta-lactam (B), Phenicol (P), Trimethoprim (R), Macrolide (M), Quinolone (Q), Sulfonamide (S), Tetracycline (T).

All isolates contained the aminoglycoside resistance gene *aac(6')-Iaa*. No genetic determinant was observed among the *S.* Infantis isolates that conferred macrolide resistance. It was also

noteworthy that all isolates possessed genetic determinants for resistance against quinolones, except for those belonging to *S.* Saintpul and *S.* I 4,[5],12:i:- serotypes. Furthermore, the *qnrB19* quinolone resistance gene (Cloeckaert and Chaslus-Dancla, 2001) was detected in only six isolates, while mutations were observed in either the *parC* (P.T57S) and/or *gyrA* (p.D87Y) genes of the remaining isolates. The extended-spectrum beta-lactamase (ESBL) gene $bla_{\text{CTX-M-65}}$ was present in all *S*. Infantis isolates. Overall, only two mutations were observed in the *nfs*A (W159*) and *nsf*B (Q137*) genes, potentially conferring resistance against nitrofurans (Table 8). All isolates possessed the genes *mdsA, mdsB, mdsC, mdtA, mdtB*, and m*dtC* that confer resistance to novobiocin, biocides, heavy metals and some β-lactams through efflux pups (Nagakubo *et al.*, 2002; Blair, Smith, *et al.*, 2015). The *mdtK* and *AcrD* genes related with multidrug efflux pumps for norfloxacin, doxorubicin, acriflavine and aminoglycosides were also found in all isolates (Rosenberg, Ma and Nikaido, 2000; Nishino, Latifi and Groisman, 2006). Besides, the *pmrG* that confers AMR and host immune evasion capabilities (Negi, Singhamahapatra and Chakravortty, 2007) was observed in all but one *S.* Amsterdam isolate. On the other hand, the *qacl* gene that encodes for a quaternary ammonium compound efflux pump (Slipski *et al.*, 2019) was detected in a single *S.* Saintpaul and one *S.* Havana isolate (Table 8).

Table 8. Genetic determinants of antimicrobial resistance (GDAMR) of *Salmonella* serotypes for each antimicrobial class at different locations of integrated poultry companies (No. of isolates (%)).

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
					***S*. Infantis**					
Aminoglycoside	*aac(6')-Iaa*	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	ant(3'')-Ia	5 (100)	-	63 (100)	-	53 (98.1)	69 (95.8)	1 (100)	-	191 (95.5)
	aph(3')-Ia	3 (60)	-	32 (50.8)	-	34 (63)	37 (51.4)	-	-	106 (53)
	aac(3)-Iva	5 (100)	-	51 (81)	-	49 (90.7)	58 (80.6)	1 (100)	-	164 (82)
	aph(4)-Ia	5 (100)	-	51 (81)	-	49 (90.7)	58 (80.6)	1 (100)	-	164 (82)
Quinolone	*parC (P.T57S)*	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	gyrA (p.D87Y)	5 (100)	-	63 (100)	-	54 (100)	72 (100)	1 (100)	-	195 (97.5)
	qnrB19	-	-	1 (1.6)	-	-	-	-	-	1 (0.5)
Nitrofurans	*nfsA (W159*)*	5 (100)	-	63 (100)	1 (25)	54 (100)	72 (100)	1 (100)	-	196 (98)
	nfsB (Q137)*	5 (100)	-	63 (100)	1 (25)	54 (100)	72 (100)	1 (100)	-	196 (98)
Tetracycline	*tet(A)*	5 (100)	-	63 (100)	-	53 (98.1)	69 (95.8)	1 (100)	-	191 (95.5)
Sulfonamide	*sul1*	5 (100)	-	63 (100)	-	53 (98.1)	69 (95.8)	1 (100)	-	191 (95.5)
Beta-lactamase	*blaCTX-M-65*	5 (100)	-	62 (98.4)	-	51 (94.4)	70 (97.2)	1 (100)	-	189 (94.5)
Phenicol	*floR*	3 (60)	-	48 (76.2)	-	49 (90.7)	54 (75)	-	-	154 (77)
Trimethoprim	*dfrA14*	5 (100)	-	46 (73)	-	47 (87)	54 (75)	1 (100)	-	153 (76.5)
Fosfomycin	*fosA3*	4 (80)	-	35 (55.6)	-	32 (59.3)	42 (58.3)	1 (100)	-	114 (57)
	fosA6	-	-	1 (1.6)	-	2 (3.7)	-	-	-	3 (1.5)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdsB	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdsC	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdtA	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdtB	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdtC	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	AcrD	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	mdtK	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)
	pmrG	5 (100)	1 (100)	63 (100)	4 (100)	54 (100)	72 (100)	1 (100)	-	200 (100)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
					***S.* Albany**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	-	-	-	-	-	1 (100)
Quinolone	*parC (P.T57S)*	-	-	1 (100)	-	-	-	-	-	1 (100)
	gyrA (p.D87Y)	-	-	1 (100)	-	-	-	-	-	1 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsB	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsC	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtA	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtB	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtC	-	-	1 (100)	-	-	-	-	-	1 (100)
	AcrD	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtK	-	-	1 (100)	-	-	-	-	-	1 (100)
	pmrG	-	-	1 (100)	-	-	-	-	-	1 (100)
					***S.* Amsterdam**					
Aminoglycoside	*aac(6')-Iaa*	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
Quinolone	*parC (P.T57S)*	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	gyrA (p.D87Y)	-	-	-	1 (5.6)	-	-	-	-	1 (5)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdsB	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdsC	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdtA	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdtB	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdtC	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	AcrD	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	mdtK	-	-	2 (100)	18 (100)	-	-	-	-	20 (100)
	pmrG	-	-	1 (50)	18 (100)	-	-	-	-	19 (95)
					***S.* Havana**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	aadA2	-	-	1 (100)	-	-	-	-	-	21 (5)
	aadA5	-	-	-	-	3 (100)	-	-	-	3 (75)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
	ant(3'')-Ia	-	-	1 (100)	-	-	-	-	-	21 (5)
Beta-lactamase	*bla*$_{CMY-2}$	-	-	1 (100)	-	-	-	-	-	21 (5)
Phenicol	*cmlA1*	-	-	1 (100)	-	-	-	-	-	21 (5)
Trimethoprim	*dfrA12*	-	-	1 (100)	-	-	-	-	-	21 (5)
	dfrA14	-	-	-	-	-	-	-	-	-
	dfrA17	-	-	-	-	3 (100)	-	-	-	3 (75)
Macrolide	*mef(B)*	-	-	1 (100)	-	-	-	-	-	21 (5)
Quinolone	*qnrB19*	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	parC (P.T57S)	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	gyrA (p.D87Y)	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
Sulfonamide	*sul2*	-	-	-	-	3 (100)	-	-	-	3 (75)
	sul3	-	-	1 (100)	-	-	-	-	-	21 (5)
Tetracycline	*tet(A)*	-	-	1 (100)	-	1 (33.3)	-	-	-	2 (50)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdsB	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdsC	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdtA	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdtB	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdtC	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	qacl	-	-	1 (100)	-	-	-	-	-	21 (5)
	AcrD	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	mdtK	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
	pmrG	-	-	1 (100)	-	3 (100)	-	-	-	4 (100)
					***S.* I 4,[5],12:i:-**					
Aminoglycoside	aac(3)-VIa_2	-	-	-	-	-	-	-	1 (100)	1 (100)
	aac(6')-Iaa	-	-	-	-	-	-	-	1 (100)	1 (100)
	ant(3'')-Ia	-	-	-	-	-	-	-	1 (100)	1 (100)
Beta-lactamase	*blaHERA-3*	-	-	-	-	-	-	-	1 (100)	1 (100)
Sulfonamide	*sul1*	-	-	-	-	-	-	-	1 (100)	1 (100)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdsB	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdsC	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdtA	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdtB	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdtC	-	-	-	-	-	-	-	1 (100)	1 (100)
	AcrD	-	-	-	-	-	-	-	1 (100)	1 (100)
	mdtK	-	-	-	-	-	-	-	1 (100)	1 (100)
	pmrG	-	-	-	-	-	-	-	1 (100)	1 (100)
					***S.* Javiana**					
Aminoglycoside	*aac(3)-IVa*	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	aac(6')-Iaa	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
Beta-lactamase	*bla*$_{\text{CMY-2}}$	-	-	-	-	-	-	1 (100)	-	1 (50)
Quinolone	*qnrB19*	-	-	-	-	-	-	1 (100)	-	1 (50)
	parC (P.T57S)	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdsB	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdsC	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdtA	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdtB	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdtC	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	AcrD	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	mdtK	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
	pmrG	-	-	1 (100)	-	-	-	1 (100)	-	2 (100)
					***S.* Liverpool**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
Quinolone	*parC (P.T57S)*	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	gyrA (p.D87Y)	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
Efflux pups for biocides, heavy	*mdsA*	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	mdsB	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
metals, and antimicrobials.	*mdsC*	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	mdtA	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	mdtB	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	mdtC	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	AcrD	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	mdtK	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
	pmrG	-	-	1 (100)	8 (100)	-	-	-	-	9 (100)
					***S.* Mbandaka**					
Aminoglycoside	*aac(6')-Iaa*	-	-	-	1 (100)	-	-	-	-	1 (100)
Quinolone	*parC (P.T57S)*	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdsA	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdsB	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdsC	-	-	-	1 (100)	-	-	-	-	1 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdtA*	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdtB	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdtC	-	-	-	1 (100)	-	-	-	-	1 (100)
	AcrD	-	-	-	1 (100)	-	-	-	-	1 (100)
	mdtK	-	-	-	1 (100)	-	-	-	-	1 (100)
	pmrG	-	-	-	1 (100)	-	-	-	-	1 (100)
					***S.* Muenchen**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	-	-	-	-	-	1 (100)
Quinolone	*parC (P.T57S)*	-	-	1 (100)	-	-	-	-	-	1 (100)
Tetracycline	*tet(A)*	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsA	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsB	-	-	1 (100)	-	-	-	-	-	1 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsC*	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtA	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtB	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtC	-	-	1 (100)	-	-	-	-	-	1 (100)
	AcrD	-	-	1 (100)	-	-	-	-	-	1 (100)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
	mdtK	-	-	1 (100)	-	-	-	-	-	1 (100)
	pmrG	-	-	1 (100)	-	-	-	-	-	1 (100)
					***S.* Saintpaul**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	ant(3'')-Ia	-	-	1 (100)	-	-	-	-	-	1 (50)
Beta-lactamase	*bla*$_{CMY-2}$	-	-	1 (100)	-	-	-	-	-	1 (50)
Phenicol	*cmlA1*	-	-	1 (100)	-	-	-	-	-	1 (50)
Trimethoprim	*dfrA12*	-	-	1 (100)	-	-	-	-	-	1 (50)
Macrolide	*mef(B)*	-	-	1 (100)	-	-	-	-	-	1 (50)
Sulfonamide	*sul3*	-	-	1 (100)	-	-	-	-	-	1 (50)
Tetracycline	*tet(A)*	-	-	1 (100)	-	-	-	-	-	1 (50)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdsB	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdsC	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdtA	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdtB	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdtC	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	qacl	-	-	1 (100)	-	-	-	-	-	1 (50)
	AcrD	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	mdtK	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
	pmrG	-	-	1 (100)	-	1 (100)	-	-	-	2 (100)
					***S.* Soerenga**					
Aminoglycoside	*aac(6')-Iaa*	-	1 (100)	-	-	-	-	-	-	1 (100)
Quinolone	*parC (P.T57S)*	-	1 (100)	-	-	-	-	-	-	1 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	1 (100)	-	-	-	-	-	-	1 (100)
	mdsB	-	1 (100)	-	-	-	-	-	-	1 (100)
	mdsC	-	1 (100)	-	-	-	-	-	-	1 (100)
	mdtA	-	1 (100)	-	-	-	-	-	-	1 (100)
	mdtB	-	1 (100)	-	-	-	-	-	-	1 (100)

Family	Gene	Caeca content	Compound feed	Overshoes	Raw feed materials	Skin after chilling	Skin after final washing	Transport paper	Turkey house	Total
	mdtC	-	1 (100)	-	-	-	-	-	-	1 (100)
	AcrD	-	1 (100)	-	-	-	-	-	-	1 (100)
	mdtK	-	1 (100)	-	-	-	-	-	-	1 (100)
	pmrG	-	1 (100)	-	-	-	-	-	-	1 (100)
					***S.* Uganda**					
Aminoglycoside	*aac(6')-Iaa*	-	-	1 (100)	-	-	-	-	-	1 (100)
Quinolone	*parC (P.T57S)*	-	-	1 (100)	-	-	-	-	-	1 (100)
Efflux pups for biocides, heavy metals, and antimicrobials.	*mdsA*	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsB	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdsC	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtA	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtB	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtC	-	-	1 (100)	-	-	-	-	-	1 (100)
	AcrD	-	-	1 (100)	-	-	-	-	-	1 (100)
	mdtK	-	-	1 (100)	-	-	-	-	-	1 (100)
	pmrG	-	-	1 (100)	-	-	-	-	-	1 (100)

4.3. Virulence genes

A total of 116 virulence genes were detected among the *Salmonella* collection (Annex 1). Integration B presented a greater diversity of serotypes and virulence genes between both integrations, but the number of isolates sequenced was also greater than integration A (Table 5). Interestingly, in both integrations the most frequent virulence patterns were pattern 37 (73.5%; 103 virulence genes) and pattern 32 (14%; 102 virulence genes) (Annex 1)

All serotypes possessed virulence genes encoding for fimbrial and nonfimbrial adherence, magnesium uptake, and secretion systems. Serotypes I4,[5],12:i:-, Infantis, Muenchen, Saintpaul, Soerenga, and Uganda contained virulence genes that promote intracellular survival in macrophages. Moreover, serotype *S.* I4,[5],12:i:- possessed genes encoding for serum resistance and the virulence-associated spv locus (Guiney and Fierer, 2011). Most *S.* Infantis isolates also contained genes encoding for iron uptake, while *S.* Javiana isolates possessed the *cdtB* typhoid toxin coding gene. Genetic determinants encoding for stress adaptation and virulence were also observed in *S.* I4,[5],12:i:- and *S.* Saintpaul isolates (Table 9).

Table 9. Virulence factors found in *Salmonella* isolates (No. of isolates (%)).

VF class	Virulence factors	Serotype											
		Albany	**Amsterdam**	**Havana**	**I 4,[5],12:i:-**	**Infantis**	**Javiana**	**Liverpool**	**Mbandaka**	**Muenchen**	**Saintpaul**	**Soerenga**	**Uganda**
Fimbrial adherence	Agf/Csg	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
	Fim	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
	Lpf	100 (1)	-	100 (4)	100 (1)	100 (200)	-	-	100 (1)	100 (1)	100 (2)	100 (1)	-
	Pef	-	-	-	100 (1)	-	-	-	-	-	-	-	-
	Ste	-	-	-	100 (1)	100 (200)	-	-	-	100 (1)	100 (2)	-	100 (1)
Macrophage inducible genes	Mig-14	-	-	-	100 (1)	100 (200)	-	-	-	100 (1)	100 (2)	100 (1)	100 (1)
Magnesium uptake	Mg2+ transport	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
Nonfimbrial adherence	MisL	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	-	100 (1)	100 (2)	100 (1)	100 (1)
	RatB	-	-	-	100 (1)	100 (200)	-	-	-	100 (1)	100 (2)	-	-
	ShdA	-	-	-	100 (1)	-	-	-	-	-	-	-	-
	SinH	-	100 (20)	-	100 (1)	100 (200)	-	-	100 (1)	100 (1)	100 (2)	-	100 (1)
Secretion system	TTSS (SPI-1 encode)	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
	TTSS (SPI-2 encode)	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
	TTSS effectors translocated	-	-	-	100 (1)	100 (200)	100 (2)	-	-	-	-	-	-

	via both systems												
	TTSS-1 translocated effectors	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
	TTSS-2 translocated effectors	100 (1)	100 (20)	100 (4)	100 (1)	100 (200)	100 (2)	100 (9)	100 (1)	100 (1)	100 (2)	100 (1)	100 (1)
Serum resistance	Rck	-	-	-	100 (1)	-	-	-	-	-	-	-	-
Spv locus	Spv	-	-	-	100 (1)	-	-	-	-	-	-	-	-
Stress adaptation	SodCI	-	-	-	100 (1)	-	-	-	-	-	100 (2)	-	-
Toxin	Typhoid toxin	-	-	-	-	-	100 (2)	-	-	-	-	-	-
Antivirulence	gifsy-2	-	-	-	100 (1)	-	-	-	-	-	100 (2)	-	-
Iron Uptake	Yersiniabactin siderophore (Escherichia)	-	-	-	-	97.5 (195)	-	-	-	-	-	-	-
	Yersiniabactin (Yersinia)	-	-	-	-	97.5 (195)	-	-	-	-	-	-	-

4.4. Genotypes

All *S.* Infantis isolates from this study belonged to ST32. Sequence type designation for the remaining serotypes is presented in Table 4. Isolates that clustered together generally originated from the same farm, and/or the same site within a specific farm. However, one genotype was isolated from different farms and production stages within integration B, suggesting that this clone can occupy multiple niches within the operation (orange cluster in Figure 4). Additionally, some clones (yellow clusters) were observed on the carcass surfaces post-final rinsing, demonstrating a propensity to survive the sanitation process in the slaughter facilities of both integrations (Figures 4 and 5). Interestingly, clones belonging to serovar Infantis were observed persisting throughout all levels of the production chain of integration A (blue cluster, Figure 4). The SNP tree analysis also revealed that some clones were distributed across multiple farms and were able to persist, ultimately contaminating carcasses in the slaughter facilities of both integrations (yellow cluster in Figure 4 and 5). Some farms delivered carcasses that were observed to be contaminated with clones only recovered from slaughter facilities within both integrations (green clusters in Figure 4 and 5). Remarkably, one *S.* Infantis genotype only isolated at slaughterhouse level, was present in carcasses originated from different farms indicating that this strain could be well adapted to the slaughterhouse environment (red cluster in Figure 5). Finally, isolates of *S.* Infantis that were detected in feed and its raw material components formed a unique cluster that possessed minimal GDAMR and the lack of pESI-like plasmids (Figure 5).

Although all the isolates from farms and slaughterhouses in both integrations are highly related, the conformed clades were limited to one or the other integration (Figure 6). Therefore, the SNP tree analysis of both companies showed that there is no cross contamination between integrations and evidence the power of this technique to trace possible outbreaks to their origin.

Principal Components analysis.

The PCA analysis showed a clear separation in two clades of isolates from feed mill and the ones from broiler farms and slaughterhouses in the PC1. Besides, the PC2 exhibiting the formation of closely related groups of isolates arranged along a line of genetic differentiation (Figure 7). The genogroups from broiler farms and slaughterhouses were composed for strains proceed from different sampling origins (raw feed materials, transport paper, overshoes, skin, etc), suggesting the existence of well-established genotypes. Such relationship could be confirmed and visualized by the neighbour-joining (NJ) tree (Figure 8).

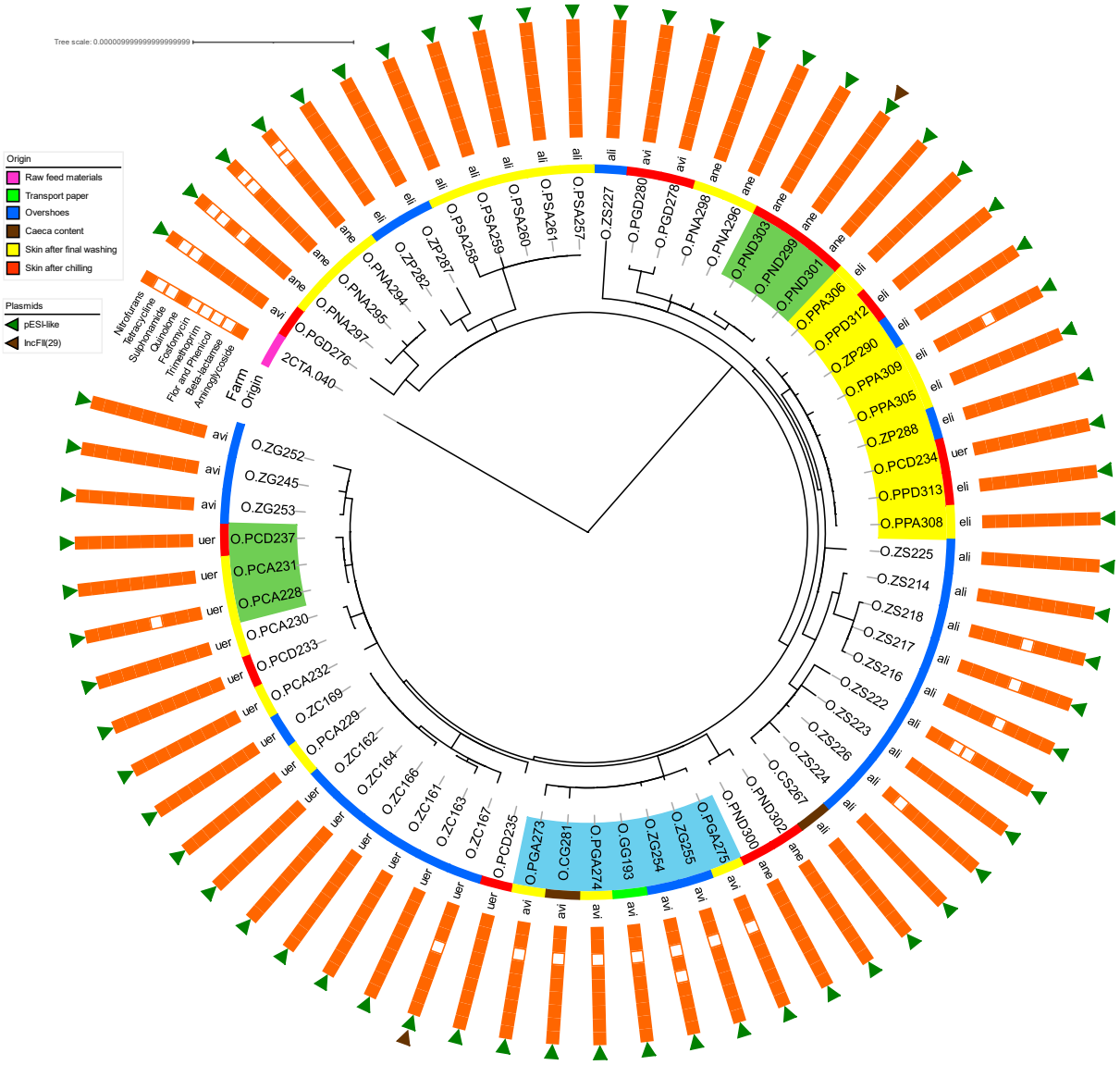


Figure 4. SNP tree analysis of *S.* Infantis isolates of integration A.

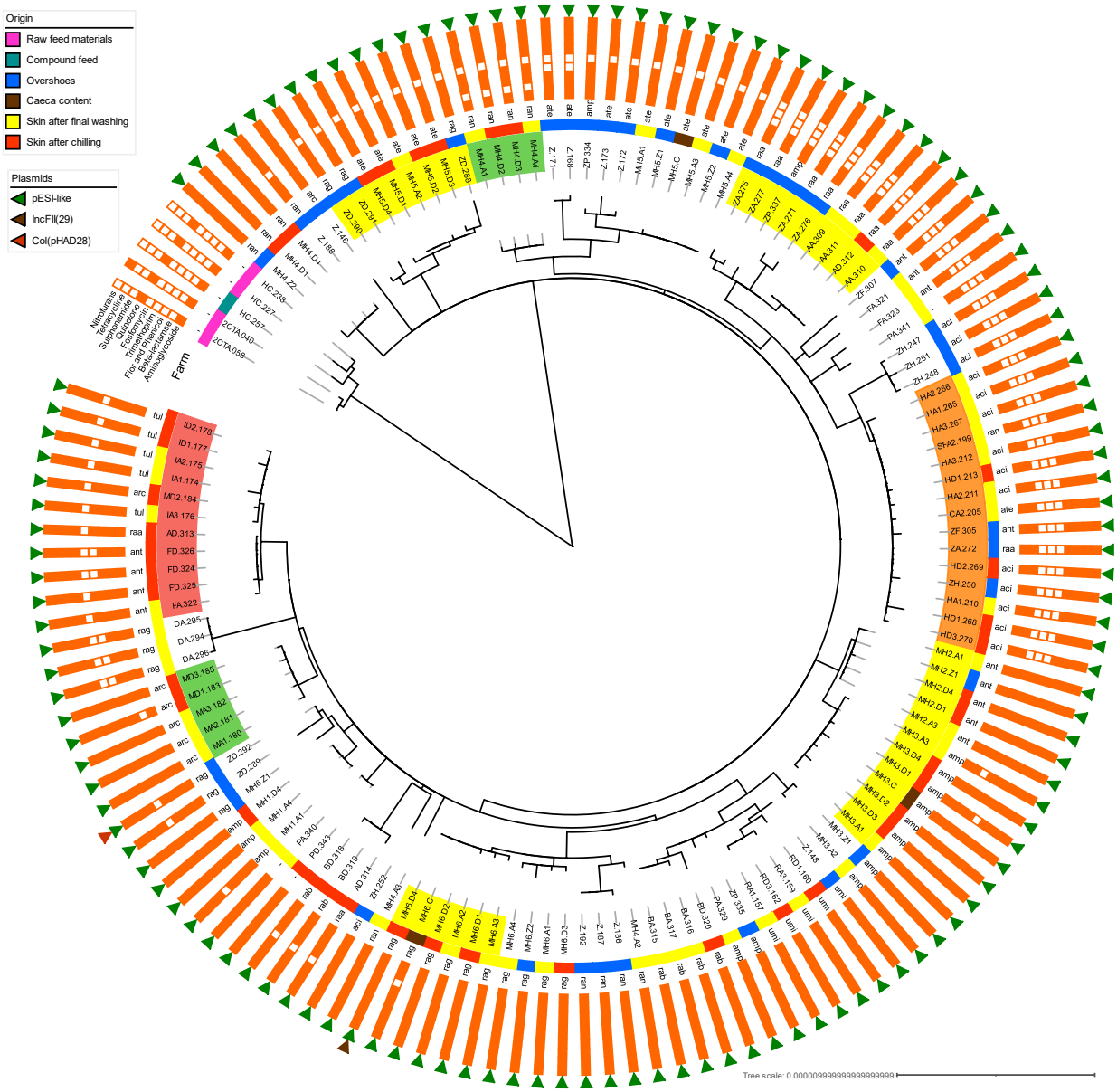


Figure 5. SNP tree analysis of *S.* Infantis isolates of integration B.

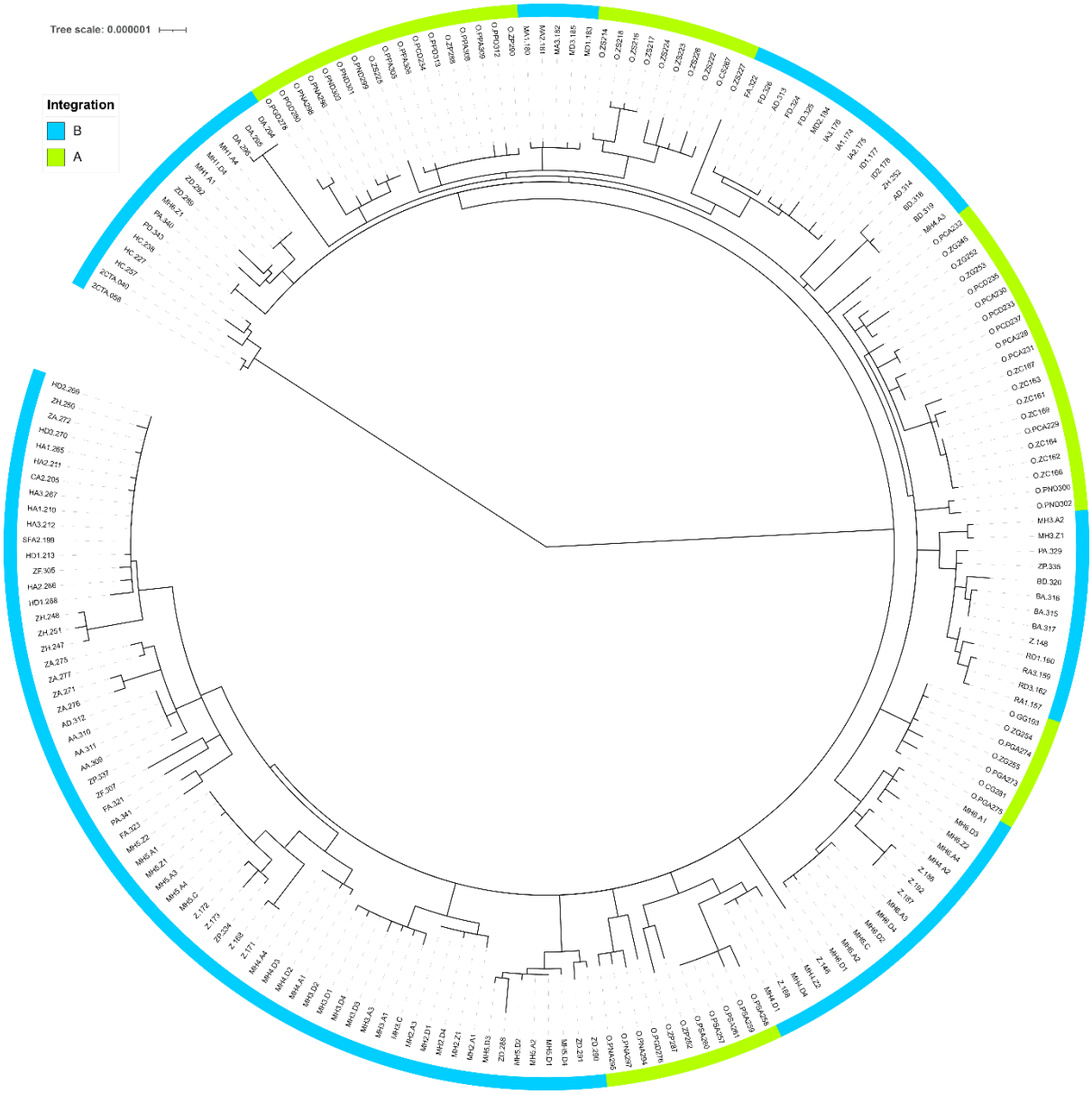


Figure 6. SNP tree analysis of *Salmonella* isolates from integration A and B.

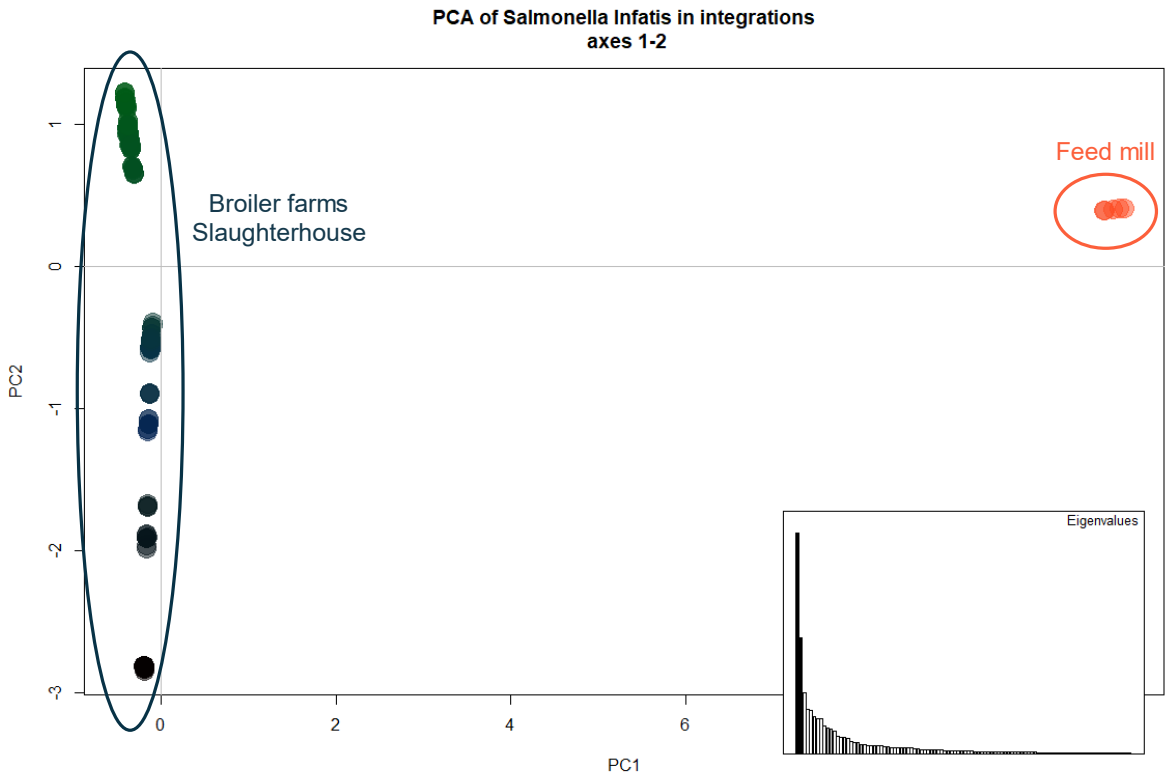


Figure 7. Principal component analysis of *S.* Infantis isolated throughout two poultry production chains.

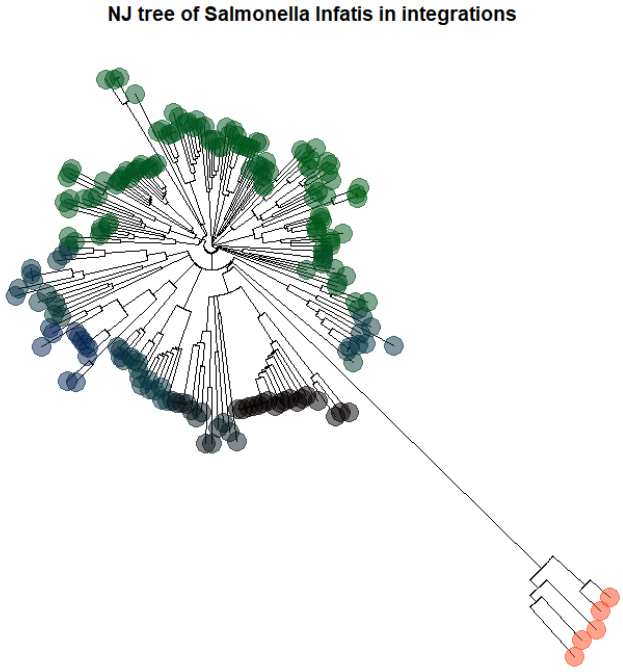


Figure 8. Neighbour-joining (NJ) tree of *S.* Infantis isolated throughout two poultry production chains.

4.5. Plasmid identification.

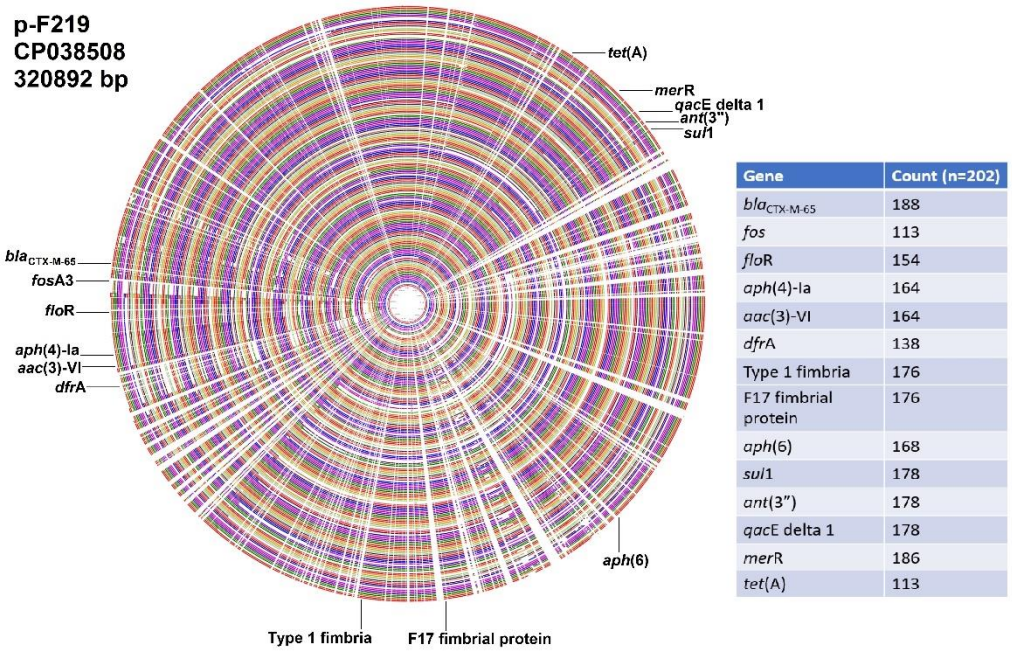
Four incompatibility groups were detected among the isolates in this study using Plasmid finder, including Col, IncFIB, IncFII, and IncI1 (Table 10). Furthermore, the map to reference analysis identified pESI-like plasmids in most (195/200) of the *S.* Infantis isolates in this study, showing homology to the reference p-F219 plasmid. In the isolates that originated in the feed mill plant, plasmids were not identified. (Figure 4).

Table 10. Presence of plasmids in each serotype.

Serovar (n)	Col(BS512)	Col(pHAD28)	Col440II	IncFIB(S)	IncFII(29)	IncFII(S)	IncI1 Alpha	pESI-like
S. Albany (1)	-	-	-	-	-	-	-	-
S. Amsterdam (20)	-	-	-	-	-	-	-	-
S. Havana (4)	-	4	-	-	-	-	4	-
S. I 4,[5],12:i:- (1)	-	-	-	1	-	1	1	-
S. Infantis (200)	-	1	-	-	3	-	-	195
S. Javiana (2)	1	1	-	-	-	-	1	-
S. Liverpool (9)	-	-	-	-	-	-	-	-
S. Mbandaka (1)	-	-	-	-	-	-	-	-
S. Muenchen (1)	-	-	-	-	-	-	-	-
S. Saintpaul (2)	-	-	-	-	-	-	1	-
S. Soerenga (1)	-	-	1	-	-	-	-	-
S. Uganda (1)	-	-	-	-	-	-	-	-
Total	1	6	1	1	3	1	7	195

Plasmid identification used by Plasmid Finder database is based on specific replicon identification.

All pESI-like plasmids presented genes associated to the success of *S.* Infantis in the poultry production environment and related to human infections. These genetic elements include: antimicrobial resistance genes *bla*$_{\text{CTX-M-65}}$, *fos*A3, *aph*(4)-Ia, *aph*(6), *aac*(3)-VI, *tet*A, *flor*R, *dfr*A, *sul*1; antiseptic resistance genes *mer* and *qac*E delta 1; and, adherence fimbria type 1 and F17 genes (Figure 9). The IncFIB(pN55391) plasmid was also identified in all *S.* Infantis isolates. However, after a map to reference verification, it was concluded that IncFIB(pN55391) plasmids were indeed pESI-like plasmids camouflaged in its chimeric structure.



Gene	Count (n=202)
bla$_{CTX-M-65}$	188
fos	113
floR	154
aph(4)-Ia	164
aac(3)-VI	164
dfrA	138
Type 1 fimbria	176
F17 fimbrial protein	176
aph(6)	168
sul1	178
ant(3")	178
qacE delta 1	178
merR	186
tet(A)	113

Figure 9. General pESI-like plasmid alignment.
PSI-like plasmids showed high similarity with the Peruvian reference. However, entire blocks are reorganized or absent in some strains.

5. Discussion

This study examined the epidemiology and population dynamics of *Salmonella* serotypes present within two poultry integrations, and to determine the presence of genetic factors that may contribute to the environmental fitness and pathogenicity of NTS, using a whole genome sequencing approach. *S.* Infantis was the most prevalent serotype in this study, thus adding additional evidence to multiple reports that have described this serovar as a global emerging pathogen in poultry production in the Americas (Valderrama *et al.*, 2014; Cunha-Neto *et al.*, 2018; Gymoese *et al.*, 2019; Lapierre *et al.*, 2020; Mejía *et al.*, 2020) , Europe (EFSA and ECDC, 2019) and Asia (Ishihara *et al.*, 2020; Li *et al.*, 2020).

In fact, the strong association between *S.* Infantis and poultry in Ecuador, and its governed territory of the Galapagos Islands, is well-described (Vinueza-Burgos *et al.*, 2016, 2019; Burnett *et al.*, 2021). Moreover, its wide distribution in both integrations highlights the propensity of *S.* Infantis to thrive in various environments. *Salmonella* Infantis has been reported to be the most common serotype in live chickens and their byproducts (Salazar *et al.*, 2019; Mejía, Vela and Zapata, 2021). Although human isolates were not included in this study, *S.* Infantis is frequently isolated from NTS diarrheal disease (Cartelle Gestal *et al.*, 2016; Mejía *et al.*, 2020; EFSA and ECDC, 2021). Furthermore, NTS outbreaks in Europe and USA have been linked to travelers returning from Ecuador and South America (Brown *et al.*, 2018; Alba *et al.*, 2020; Bokhary *et al.*, 2021). This highlights the need to implement strong monitoring and control measures to mitigate the presence of this pathogen.

The population dynamics of *Salmonella* in the production chain of broiler chickens has been investigated using various methods including serotyping (Foley *et al.*, 2011), ribotyping and

PFGE (Liebana *et al.*, 2001; CDC, 2016; Vinueza-Burgos *et al.*, 2019). Despite the discriminatory power of PFGE, this method has clear limitations when used to analyze highly similar bacterial genomes as is the case of *S.* Infantis. Moreover, the typification by MLST shows to be of insufficient resolution when looking for the transmission of specific genotypes throughout the broiler production chain. In fact, all *S.* Infantis isolates in this study belonged to ST32 which is the most prevalent ST of this serotype (Achtman *et al.*, 2020). It is here where a WGS approach takes place for the analysis of the transmission of *Salmonella* genotypes in integrated poultry companies, since WGS gives a level of resolution that cannot be reached with other techniques.

For example, the red cluster in Figure 5 shows that a single genotype, only found at slaughterhouse level, contaminates carcasses from different farms. The fact that this genotype was not isolated in previous stages of the production chain, could suggest that this clone is well adapted to the slaughterhouse environment (e.g., forming biofilms) and could provoke cross-contamination events of carcasses at this level. Furthermore, our analysis shows that *Salmonella* genotypes originated in specific farms can enter into the slaughter line and reach carcasses of different farms (orange and yellow clusters in Figures 4 and 5). These facts highlight the role of the slaughter process as source of *Salmonella* contamination of broiler carcasses (Rasschaert *et al.*, 2006; Rouger, Tresse and Zagorec, 2017).

Interestingly, isolates originating from the feed mill plant were observed to be the most genetically unique, when compared to the remaining collection. Moreover, these isolates presented fewer GDAMR. This observation has been reported before and could be explained by the selective pressure caused by the usage of antibiotics at farm level (Vinueza-Burgos *et al.*, 2016; Villagómez, Logacho and Vinueza, 2017). Besides, this fact could demonstrate that *Salmonella* strains originated in raw materials and feed compound are not important players in the epidemiology of this bacteria in farther stages of broiler production.

On the other hand, the isolate originated in transport paper of one-day-old chicken showed to be highly related to the ones originated in farms and slaughterhouse of integration A (blue cluster). This observation reveals that the production system of one-day-old chickens could play an important role in the epidemiology of *S.* Infantis in the poultry production. Therefore, earlier stages of production (e.g., breeders, hatchery, transport, etc.) should be included when planning surveillance programs for *Salmonella*. Moreover, the data presented here show an evident clustering of isolates in each integration (supported by PCA analysis), evidencing the suitability of this technique to track pathogens up to their origin and identify hotspots of *Salmonella* contamination in the broiler production chain (Figure 6).

Our observations suggest that *Salmonella* isolates are able to persist, despite sanitation steps at various points of the production chain. Unsurprisingly, genetic determinants capable of conferring resistance against disinfectants, including quaternary ammonium compounds, as well as heavy metal tolerance, were observed in multiple isolates.

The ability of *S.* Infantis to persist in food and food processing environments and to establish successful infections in humans has resulted in its rise as an emerging pathogen. Some genetic studies suggested that *S.* Infantis isolates possess important GDAMR and virulence-associated genes that contribute to its ability to adapt and cause successful infections (Acar *et al.*, 2019; Bogomazova *et al.*, 2020; Proietti *et al.*, 2020). Most *S.* Infantis isolates detected in this study possessed GDAMR that could potentially confer resistance to first line antibiotics used to treat humans NTS infections (beta lactams, quinolones, and sulfonamides) (Onwuezobe, Oshun and Odigwe, 2012). A similar observation was made for the other serovars; however, phenotypic studies are needed to confirm the genome predicted resistance. Nonetheless, studies have been

conducted in Ecuador that correlate the presence of GDAMR with phenotypic resistance in *S.* Infantis (Villagómez, Logacho and Vinueza, 2017; Vinueza-Burgos *et al.*, 2019; Mejía *et al.*, 2020). In fact, it has been shown a high correlation of the presence of GDAMR and resistant phenotypes (Bortolaia *et al.*, 2020).

Virulence genes including the plasmid-encoded *spv* have been associated with increased virulence in *Salmonella* isolated from clinically ill patients (Guiney *et al.*, 1995; Guiney and Fierer, 2011) and their presence in isolates from this study represents a potential risk to final consumers (Tate *et al.*, 2017; EFSA and ECDC, 2019; Tyson *et al.*, 2021).

In this study the pESI-like megaplasmid was identified in almost all *S.* Infantis isolates throughout the production chain of the two integrations. In fact, 95% of them harbored the *bla*$_{\text{CTX-M-65}}$ gene that confers resistance to Third Generation Cephalosporins (3GC). Although 3GC are not used in poultry production, the presence of the *bla*$_{\text{CTX-M-65}}$ gene could be explained by a co-selection of resistance genes to antibiotics that are commonly utilized in broiler production (Franco *et al.*, 2015; Pal *et al.*, 2015; McMillan, Jackson and Frye, 2020). This is the case of the genes *fos*A3 (fosfomycin), *tet*A (tetracycline), *dfr*A (trimethoprim), *sul*1 (sulfonamide); and the antiseptic resistance gene *qac*E delta 1 found in the analyzed pESI-like plasmids. However, more research is needed to demonstrate this hypothesis in pESI-like plasmid of *S.* Infantis.

These genes have also been described in *S.* Infantis isolated from broiler production in Peru (Vallejos-Sánchez *et al.*, 2019). The presence of *bla*$_{\text{CTX-M-65}}$ in these plasmids could be implicated with their permanence in environments with β-lactam antibiotics pressure (e.g contaminated water sources or soil). The high rates of pESI-like plasmids in 3GC-resistant *S.* Infantis originated in poultry environments have been reported in several studies worldwide (Franco *et al.*, 2015; Alba *et al.*, 2020; Bogomazova *et al.*, 2020; García-Soto *et al.*, 2020; McMillan *et al.*, 2020; Kürekci *et al.*, 2021). Although the dynamics of pESI-like plasmids remains largely unknown, it has been claimed that the specificity of pESI-like plasmids in *S.* Infantis could be associated with the inhibition of conjugation with other *Salmonella* serogroups in the chicken gut, mainly mediated by temperature and the presence of bile salts (Thomas and Nielsen, 2005; García-Soto *et al.*, 2020). Additionally, the inhibition of self-transmission of these plasmids to *S.* Typhimurium and *Escherichia coli* has been demonstrated in laboratory (Aviv *et al.*, 2014; Aviv, Rahav and Gal-Mor, 2016). However, more studies are needed to characterize the ecological barriers for intra- and inter-specific transmission of pESI-like plasmids to other bacteria in the poultry industry.

Another important feature of pESI-like plasmids found in this study is the presence of several fimbriae genes (type 1 and F17 fimbria), that could increase the capacity of attachment to epithelial cells of this bacteria (Aviv *et al.*, 2014; Aviv, Rahav and Gal-Mor, 2016). Altogether, these factors could represent major drivers for the increase of human infections caused by ESBL-producing *S.* Infantis (McMillan *et al.*, 2020). Therefore, the study of the molecular epidemiology of pESI-like plasmids should be included as a part of the surveillance programs to reduce the dissemination of this microorganism in the food chain.

It has been reported that pESI-like plasmids display chimeric characteristics that can cause its description as IncFIB plasmid or its variants in other studies (Aviv *et al.*, 2014; García-Soto *et al.*, 2020; Burnett *et al.*, 2021). For this reason, this characteristic should be considered when reporting findings of the molecular epidemiology of this plasmid. It is also worth to mention that pESI-like plasmids can enhance the fitness of specific *S.* Infantis strains, displacing other genotypes in niches within the poultry industry (Bogomazova *et al.*, 2020).

In this study we demonstrate the usefulness of a WGS approach to have an in-depth understanding of the epidemiology of *Salmonella* in integrated poultry companies. This kind of analysis can help to implement and evaluate interventions aiming to control *Salmonella* in the broiler production chain. Moreover, we report that *S.* Infantis is the main *Salmonella* serotype in studied integrations and that the pESI-like plasmids found in these isolates harbor important resistance and virulence genes.

6. Recommendations

- To perform a detailed analysis of *Salmonella* strains originated in hotspots within integrated poultry companies in order to design intervention plans to control this pathogen in the broilers production chain.
- To put in place a surveillance plan in integrated poultry companies based on WGS to gain insights on results of interventions aiming to control *Salmonella* in the production of broilers.
- To use WGS to analyze the coevolution of antimicrobial resistance determinants in mobile structures.

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8. ANNEXES

Annex 1. List of isolates sequenced in the research (BioProject PRJNA377900).

Nº	Name	BioSample	Enterobase ID	Origin	Integration	Serovar	ST	Virulence genes (Nº)	Virulence Pattern
1	O.PA115	SAMN10451620	SAL_ZA7891AA	Raw feed materials	A	Mbandaka	413	70	33
2	O.ZC161	SAMN10451618	SAL_ZA7893AA	Overshoes	A	Infantis	32	103	37
3	O.ZC162	SAMN10451619	SAL_ZA7896AA	Overshoes	A	Infantis	32	103	37
4	O.ZC163	SAMN10451654	SAL_ZA7894AA	Overshoes	A	Infantis	32	103	37
5	O.ZC164	SAMN10451670	SAL_ZA7899AA	Overshoes	A	Infantis	32	103	37
6	O.ZC166	SAMN10451671	SAL_ZA7897AA	Overshoes	A	Infantis	32	102	11
7	O.ZC167	SAMN10451667	SAL_ZA7898AA	Overshoes	A	Infantis	32	103	37
8	O.ZC169	SAMN10451604	SAL_ZA7900AA	Overshoes	A	Infantis	32	103	37
9	O.GG193	SAMN10451680	SAL_ZA7901AA	Transport paper	A	Infantis	32	103	37
10	O.ZS214	SAMN10451683	SAL_ZA7903AA	Overshoes	A	Infantis	32	103	37
11	O.ZS216	SAMN10451661	SAL_ZA7902AA	Overshoes	A	Infantis	32	102	33
12	O.ZS217	SAMN10451609	SAL_ZA7904AA	Overshoes	A	Infantis	32	101	18
13	O.ZS218	SAMN10451626	SAL_ZA7906AA	Overshoes	A	Infantis	32	103	37
14	O.ZS222	SAMN10451668	SAL_ZA7908AA	Overshoes	A	Infantis	32	103	37
15	O.ZS223	SAMN10451666	SAL_ZA7905AA	Overshoes	A	Infantis	32	101	14
16	O.ZS224	SAMN10451660	SAL_ZA7907AA	Overshoes	A	Infantis	32	100	12
17	O.ZS225	SAMN15588449	SAL_ZA7910AA	Overshoes	A	Infantis	32	102	32
18	O.ZS226	SAMN10589485	SAL_ZA7909AA	Overshoes	A	Infantis	32	102	13
19	O.ZS227	SAMN10521941	SAL_ZA7597AA	Overshoes	A	Infantis	32	103	37
20	O.PCA228	SAMN10521940	SAL_ZA7599AA	Skin after final washing	A	Infantis	32	103	37
21	O.PCA229	SAMN10588588	SAL_ZA7601AA	Skin after final washing	A	Infantis	32	101	10
22	O.PCA230	SAMN10521939	SAL_ZA7603AA	Skin after final washing	A	Infantis	32	101	18
23	O.PCA231	SAMN10589417	SAL_ZA7602AA	Skin after final washing	A	Infantis	32	103	37
24	O.PCA232	SAMN10521932	SAL_ZA7604AA	Skin after final washing	A	Infantis	32	102	32
25	O.PCD233	SAMN10589412	SAL_ZA7605AA	Skin after chilling	A	Infantis	32	102	13
26	O.PCD234	SAMN10521933	SAL_ZA7606AA	Skin after chilling	A	Infantis	32	103	37
27	O.PCD235	SAMN10521934	SAL_ZA7609AA	Skin after chilling	A	Infantis	32	103	37
28	O.PCD237	SAMN10589411	SAL_ZA7607AA	Skin after chilling	A	Infantis	32	103	37
29	O.ZG245	SAMN10521942	SAL_ZA7610AA	Overshoes	A	Infantis	32	103	37
30	O.ZG246	SAMN10521946	SAL_ZA7608AA	Overshoes	A	Amsterdam	2090	77	26
31	O.ZG247	SAMN10521947	SAL_ZA7612AA	Overshoes	A	Amsterdam	2090	77	26
32	O.ZG252	SAMN10521957	SAL_ZA7611AA	Overshoes	A	Infantis	32	103	37
33	O.ZG253	SAMN10521905	SAL_ZA7614AA	Overshoes	A	Infantis	32	103	37
34	O.ZG254	SAMN10521906	SAL_ZA7613AA	Overshoes	A	Infantis	32	103	37
35	O.ZG255	SAMN10521951	SAL_ZA7616AA	Overshoes	A	Infantis	32	103	37
36	O.PSA257	SAMN10521943	SAL_ZA7615AA	Skin after final washing	A	Infantis	32	102	32
37	O.PSA258	SAMN10521944	SAL_ZA7617AA	Skin after final washing	A	Infantis	32	103	37
38	O.PSA259	SAMN10521945	SAL_ZA7618AA	Skin after final washing	A	Infantis	32	103	37
39	O.PSA260	SAMN10521908	SAL_ZA7619AA	Skin after final washing	A	Infantis	32	103	37

40	O.PSA261	SAMN10521909	SAL_ZA7620AA	Skin after final washing	A	Infantis	32	103	37
41	O.CS267	SAMN10521900	SAL_ZA7621AA	Caeca content	A	Infantis	32	103	37
42	O.PGA273	SAMN10521914	SAL_ZA7622AA	Skin after final washing	A	Infantis	32	103	37
43	O.PGA274	SAMN10521916	SAL_ZA7623AA	Skin after final washing	A	Infantis	32	103	37
44	O.PGA275	SAMN10521926	SAL_ZA7624AA	Skin after final washing	A	Infantis	32	103	37
45	O.PGD276	SAMN10589423	SAL_ZA7625AA	Skin after chilling	A	Infantis	32	103	37
46	O.PGD278	SAMN10589421	SAL_ZA7629AA	Skin after chilling	A	Infantis	32	103	37
47	O.PGD280	SAMN10521927	SAL_ZA7627AA	Skin after chilling	A	Infantis	32	103	37
48	O.CG281	SAMN10521901	SAL_ZA7626AA	Caeca content	A	Infantis	32	102	32
49	O.ZP282	SAMN10521910	SAL_ZA7628AA	Overshoes	A	Infantis	32	102	32
50	O.ZP287	SAMN10521922	SAL_ZA7630AA	Overshoes	A	Infantis	32	103	37
51	O.ZP288	SAMN10521923	SAL_ZA7631AA	Overshoes	A	Infantis	32	103	37
52	O.ZP290	SAMN10521938	SAL_ZA7632AA	Overshoes	A	Infantis	32	103	37
53	O.PNA294	SAMN10589462	SAL_ZA7634AA	Skin after final washing	A	Infantis	32	103	37
54	O.PNA295	SAMN10521920	SAL_ZA7633AA	Skin after final washing	A	Infantis	32	103	37
55	O.PNA296	SAMN10589457	SAL_ZA7635AA	Skin after final washing	A	Infantis	32	103	37
56	O.PNA297	SAMN09850700	SAL_ZA7636AA	Skin after final washing	A	Infantis	32	103	37
57	O.PNA298	SAMN10521921	SAL_ZA7637AA	Skin after final washing	A	Infantis	32	103	37
58	O.PND299	SAMN10589436	SAL_ZA7638AA	Skin after chilling	A	Infantis	32	103	37
59	O.PND300	SAMN10589447	SAL_ZA7639AA	Skin after chilling	A	Infantis	32	101	18
60	O.PND301	SAMN10521912	SAL_ZA7641AA	Skin after chilling	A	Infantis	32	103	37
61	O.PND302	SAMN10521915	SAL_ZA7640AA	Skin after chilling	A	Infantis	32	103	37
62	O.PND303	SAMN10521955	SAL_ZA7642AA	Skin after chilling	A	Infantis	32	103	37
63	O.PPA305	SAMN10521904	SAL_ZA7643AA	Skin after final washing	A	Infantis	32	103	37
64	O.PPA306	SAMN10521911	SAL_ZA7644AA	Skin after final washing	A	Infantis	32	103	37
65	O.PPA308	SAMN10521919	SAL_ZA7645AA	Skin after final washing	A	Infantis	32	103	37
66	O.PPA309	SAMN10589446	SAL_ZA7646AA	Skin after final washing	A	Infantis	32	103	37
67	O.PPD312	SAMN10589461	SAL_ZA7647AA	Skin after chilling	A	Infantis	32	103	37
68	O.PPD313	SAMN10521930	SAL_ZA7648AA	Skin after chilling	A	Infantis	32	103	37
69	2CTA.022	SAMN12108727	SAL_ZA7649AA	Raw feed materials	B	Amsterdam	2090	75	28
70	2CTA.040	SAMN12108761	SAL_ZA7650AA	Raw feed materials	B	Infantis	32	101	35
71	2CTA.056	SAMN10521949	SAL_ZA7651AA	Raw feed materials	B	Liverpool	1959	75	29
72	2CTA.058	SAMN09850715	SAL_ZA7652AA	Raw feed materials	B	Infantis	32	100	30
73	2CTA.088	SAMN12108834	SAL_ZA7654AA	Raw feed materials	B	Liverpool	1959	76	27
74	2CTA.119	SAMN12108837	SAL_ZA7653AA	Compound feed	B	Soerenga	1659	76	30
75	2CTA.159	SAMN12108838	SAL_ZA7655AA	Raw feed materials	B	Liverpool	1959	76	27
76	2CTA.170	SAMN12108825	SAL_ZA7656AA	Raw feed materials	B	Liverpool	1959	75	29
77	2CTP.0017	SAMN09850713	SAL_ZA7657AA	Turkey house	B	I 4,[5],12:i:-	19	118	1
78	2CTP.022	SAMN09850304	SAL_ZA7658AA	Transport paper	B	Javiana	1674	70	32
79	AA.309	SAMN15588412	SAL_ZA7799AA	Skin after final washing	B	Infantis	32	103	37
80	AA.310	SAMN15588452	SAL_ZA7801AA	Skin after final washing	B	Infantis	32	102	32
81	AA.311	SAMN10397013	SAL_ZA7800AA	Skin after final washing	B	Infantis	32	103	37
82	AD.312	SAMN10397020	SAL_ZA7802AA	Skin after chilling	B	Infantis	32	102	32

83	AD.313	SAMN15588350	SAL_ZA7803AA	Skin after chilling	B	Infantis	32	103	37
84	AD.314	SAMN10397018	SAL_ZA7805AA	Skin after chilling	B	Infantis	32	102	32
85	BA.315	SAMN15588454	SAL_ZA7806AA	Skin after final washing	B	Infantis	32	103	37
86	BA.316	SAMN10416364	SAL_ZA7804AA	Skin after final washing	B	Infantis	32	102	32
87	BA.317	SAMN15588391	SAL_ZA7808AA	Skin after final washing	B	Infantis	32	103	37
88	BD.318	SAMN10416372	SAL_ZA7807AA	Skin after chilling	B	Infantis	32	103	37
89	BD.319	SAMN15588457	SAL_ZA7809AA	Skin after chilling	B	Infantis	32	103	37
90	BD.320	SAMN10416390	SAL_ZA7810AA	Skin after chilling	B	Infantis	32	102	32
91	CA2.205	SAMN15588393	SAL_ZA7752AA	Skin after final washing	B	Infantis	32	103	37
92	DA.294	SAMN15588365	SAL_ZA7792AA	Skin after final washing	B	Infantis	32	103	37
93	DA.295	SAMN15588349	SAL_ZA7793AA	Skin after final washing	B	Infantis	32	103	37
94	DA.296	SAMN15588387	SAL_ZA7796AA	Skin after final washing	B	Infantis	32	103	37
95	DD.299	SAMN10397008	SAL_ZA7794AA	Skin after chilling	B	Saintpaul	50	106	2
96	FA.321	SAMN15588348	SAL_ZA7812AA	Skin after final washing	B	Infantis	32	103	37
97	FA.322	SAMN10416385	SAL_ZA7811AA	Skin after final washing	B	Infantis	32	103	37
98	FA.323	SAMN10416365	SAL_ZA7813AA	Skin after final washing	B	Infantis	32	103	37
99	FD.324	SAMN15588461	SAL_ZA7815AA	Skin after chilling	B	Infantis	32	102	32
100	FD.325	SAMN15588459	SAL_ZA7816AA	Skin after chilling	B	Infantis	32	103	37
101	FD.326	SAMN15588402	SAL_ZA7814AA	Skin after chilling	B	Infantis	32	103	37
102	HA1.210	SAMN10397010	SAL_ZA7753AA	Skin after final washing	B	Infantis	32	103	37
103	HA1.265	SAMN15588462	SAL_ZA7774AA	Skin after final washing	B	Infantis	32	103	37
104	HA2.211	SAMN10397005	SAL_ZA7754AA	Skin after final washing	B	Infantis	32	103	37
105	HA2.266	SAMN15588464	SAL_ZA7775AA	Skin after final washing	B	Infantis	32	102	32
106	HA3.212	SAMN15588460	SAL_ZA7755AA	Skin after final washing	B	Infantis	32	99	22
107	HA3.267	SAMN10741176	SAL_ZA7773AA	Skin after final washing	B	Infantis	32	103	37
108	HC.086	SAMN10524046	SAL_ZA7709AA	Raw feed materials	B	Amsterdam	2090	77	26
109	HC.087	SAMN10524049	SAL_ZA7708AA	Raw feed materials	B	Amsterdam	2090	77	26
110	HC.106	SAMN12108835	SAL_ZA7710AA	Raw feed materials	B	Amsterdam	2090	76	28
111	HC.111	SAMN15588453	SAL_ZA7712AA	Raw feed materials	B	Liverpool	1959	76	27
112	HC.128	SAMN10524043	SAL_ZA7711AA	Raw feed materials	B	Liverpool	1959	76	27
113	HC.130	SAMN10524048	SAL_ZA7718AA	Raw feed materials	B	Amsterdam	2090	77	26
114	HC.132	SAMN15588409	SAL_ZA7719AA	Raw feed materials	B	Amsterdam	2090	76	28
115	HC.155	SAMN20119337	SAL_ZA7725AA	Raw feed materials	B	Liverpool	1959	76	27
116	HC.219	SAMN15588463	SAL_ZA7756AA	Raw feed materials	B	Amsterdam	2090	76	28
117	HC.220	SAMN10397009	SAL_ZA7758AA	Raw feed materials	B	Amsterdam	2090	77	26
118	HC.227	SAMN10397011	SAL_ZA7759AA	Raw feed materials	B	Infantis	32	100	19
119	HC.228	SAMN15588467	SAL_ZA7760AA	Raw feed materials	B	Amsterdam	2090	77	26
120	HC.232	SAMN10576340	SAL_ZA7762AA	Raw feed materials	B	Amsterdam	2090	77	26
121	HC.238	SAMN10741164	SAL_ZA7761AA	Raw feed materials	B	Infantis	32	100	30
122	HC.245	SAMN20157054	SAL_ZA7763AA	Raw feed materials	B	Amsterdam	2090	77	26
123	HC.246	SAMN10524037	SAL_ZA7765AA	Raw feed materials	B	Amsterdam	2090	77	26
124	HC.255	SAMN10741169	SAL_ZA7772AA	Raw feed materials	B	Amsterdam	2090	77	26
125	HC.257	SAMN10349057	SAL_ZA7770AA	Compound feed	B	Infantis	32	101	35

126	HC.264	SAMN15588397	SAL_ZA7771AA	Raw feed materials	B	Liverpool	1959	75	31
127	HC.285	SAMN10349366	SAL_ZA7782AA	Raw feed materials	B	Amsterdam	2090	76	28
128	HC.304	SAMN10397007	SAL_ZA7795AA	Raw feed materials	B	Amsterdam	2090	77	26
129	HD1.213	SAMN10397014	SAL_ZA7757AA	Skin after chilling	B	Infantis	32	103	37
130	HD1.268	SAMN15588407	SAL_ZA7777AA	Skin after chilling	B	Infantis	32	103	37
131	HD2.269	SAMN15588466	SAL_ZA7776AA	Skin after chilling	B	Infantis	32	103	37
132	HD3.270	SAMN15588404	SAL_ZA7779AA	Skin after chilling	B	Infantis	32	103	37
133	HS.126	SAMN10524042	SAL_ZA7713AA	Raw feed materials	B	Amsterdam	2090	77	26
134	HS.133	SAMN15588465	SAL_ZA7715AA	Raw feed materials	B	Amsterdam	2090	76	28
135	HS.260	SAMN10524044	SAL_ZA7717AA	Raw feed materials	B	Amsterdam	2090	77	26
136	IA1.174	SAMN10382439	SAL_ZA7734AA	Skin after final washing	B	Infantis	32	103	37
137	IA2.175	SAMN10397157	SAL_ZA7736AA	Skin after final washing	B	Infantis	32	102	32
138	IA3.176	SAMN10349368	SAL_ZA7737AA	Skin after final washing	B	Infantis	32	102	26
139	ID1.177	SAMN20090802	SAL_ZA7738AA	Skin after chilling	B	Infantis	32	103	37
140	ID2.178	SAMN20090797	SAL_ZA7739AA	Skin after chilling	B	Infantis	32	103	37
141	MA1.180	SAMN15588385	SAL_ZA7742AA	Skin after final washing	B	Infantis	32	103	37
142	MA2.181	SAMN10349362	SAL_ZA7740AA	Skin after final washing	B	Infantis	32	103	37
143	MA3.182	SAMN15588388	SAL_ZA7741AA	Skin after final washing	B	Infantis	32	102	21
144	MD1.183	SAMN15588388	SAL_ZA7744AA	Skin after chilling	B	Infantis	32	103	37
145	MD2.184	SAMN10397015	SAL_ZA7743AA	Skin after chilling	B	Infantis	32	100	16
146	MD3.185	SAMN10397012	SAL_ZA7745AA	Skin after chilling	B	Infantis	32	103	37
147	MH1.A1	SAMN09850303	SAL_ZA7659AA	Skin after final washing	B	Infantis	32	103	37
148	MH1.A4	SAMN09850699	SAL_ZA7660AA	Skin after final washing	B	Infantis	32	103	37
149	MH1.D4	SAMN09850696	SAL_ZA7661AA	Skin after chilling	B	Infantis	32	102	32
150	MH2.A1	SAMN12108836	SAL_ZA7663AA	Skin after final washing	B	Infantis	32	103	37
151	MH2.A3	SAMN09850709	SAL_ZA7664AA	Skin after final washing	B	Infantis	32	103	37
152	MH2.D1	SAMN09850706	SAL_ZA7665AA	Skin after chilling	B	Infantis	32	103	37
153	MH2.D4	SAMN09850716	SAL_ZA7666AA	Skin after chilling	B	Infantis	32	103	37
154	MH2.Z1	SAMN09850694	SAL_ZA7662AA	Overshoes	B	Infantis	32	103	37
155	MH3.A1	SAMN09907451	SAL_ZA7670AA	Skin after final washing	B	Infantis	32	103	37
156	MH3.A2	SAMN09907452	SAL_ZA7671AA	Skin after final washing	B	Infantis	32	103	37
157	MH3.A3	SAMN09907449	SAL_ZA7673AA	Skin after final washing	B	Infantis	32	103	37
158	MH3.C	SAMN09907453	SAL_ZA7669AA	Caeca content	B	Infantis	32	103	37
159	MH3.D1	SAMN09907450	SAL_ZA7674AA	Skin after chilling	B	Infantis	32	103	37
160	MH3.D2	SAMN09907459	SAL_ZA7672AA	Skin after chilling	B	Infantis	32	102	32
161	MH3.D3	SAMN09907460	SAL_ZA7676AA	Skin after chilling	B	Infantis	32	103	37
162	MH3.D4	SAMN09907454	SAL_ZA7677AA	Skin after chilling	B	Infantis	32	102	32
163	MH3.Z1	SAMN09907456	SAL_ZA7667AA	Overshoes	B	Infantis	32	102	32
164	MH3.Z2	SAMN09907448	SAL_ZA7668AA	Overshoes	B	Uganda	684	89	23
165	MH4.A1	SAMN12108797	SAL_ZA7678AA	Skin after final washing	B	Infantis	32	103	37
166	MH4.A2	SAMN09907465	SAL_ZA7679AA	Skin after final washing	B	Infantis	32	103	37
167	MH4.A3	SAMN09907463	SAL_ZA7681AA	Skin after final washing	B	Infantis	32	102	32
168	MH4.A4	SAMN09907464	SAL_ZA7680AA	Skin after final washing	B	Infantis	32	103	37

169	MH4.D1	SAMN10576341	SAL_ZA7682AA	Skin after chilling	B	Infantis	32	103	37
170	MH4.D2	SAMN12108824	SAL_ZA7683AA	Skin after chilling	B	Infantis	32	103	37
171	MH4.D3	SAMN10576342	SAL_ZA7686AA	Skin after chilling	B	Infantis	32	103	37
172	MH4.D4	SAMN12108827	SAL_ZA7684AA	Skin after chilling	B	Infantis	32	103	37
173	MH4.Z2	SAMN09907455	SAL_ZA7675AA	Overshoes	B	Infantis	32	103	37
174	MH5.A1	SAMN10576334	SAL_ZA7689AA	Skin after final washing	B	Infantis	32	103	37
175	MH5.A2	SAMN10576335	SAL_ZA7690AA	Skin after final washing	B	Infantis	32	103	37
176	MH5.A3	SAMN10576329	SAL_ZA7691AA	Skin after final washing	B	Infantis	32	103	37
177	MH5.A4	SAMN10576330	SAL_ZA7692AA	Skin after final washing	B	Infantis	32	102	32
178	MH5.C	SAMN10576331	SAL_ZA7688AA	Caeca content	B	Infantis	32	103	37
179	MH5.D1	SAMN10576333	SAL_ZA7693AA	Skin after chilling	B	Infantis	32	101	24
180	MH5.D2	SAMN10576339	SAL_ZA7694AA	Skin after chilling	B	Infantis	32	103	37
181	MH5.D3	SAMN10576344	SAL_ZA7695AA	Skin after chilling	B	Infantis	32	102	32
182	MH5.D4	SAMN10576337	SAL_ZA7696AA	Skin after chilling	B	Infantis	32	103	37
183	MH5.Z1	SAMN10576338	SAL_ZA7687AA	Overshoes	B	Infantis	32	101	18
184	MH5.Z2	SAMN10576343	SAL_ZA7685AA	Overshoes	B	Infantis	32	103	37
185	MH6.A1	SAMN12108802	SAL_ZA7701AA	Skin after final washing	B	Infantis	32	103	37
186	MH6.A2	SAMN12108809	SAL_ZA7700AA	Skin after final washing	B	Infantis	32	103	37
187	MH6.A3	SAMN12108795	SAL_ZA7704AA	Skin after final washing	B	Infantis	32	103	37
188	MH6.A4	SAMN10524039	SAL_ZA7702AA	Skin after final washing	B	Infantis	32	103	37
189	MH6.C	SAMN10576336	SAL_ZA7699AA	Caeca content	B	Infantis	32	103	37
190	MH6.D1	SAMN10524047	SAL_ZA7703AA	Skin after chilling	B	Infantis	32	103	37
191	MH6.D2	SAMN10524050	SAL_ZA7705AA	Skin after chilling	B	Infantis	32	102	32
192	MH6.D3	SAMN10524036	SAL_ZA7706AA	Skin after chilling	B	Infantis	32	103	37
193	MH6.D4	SAMN10524045	SAL_ZA7707AA	Skin after chilling	B	Infantis	32	102	32
194	MH6.Z1	SAMN20119335	SAL_ZA7697AA	Overshoes	B	Infantis	32	103	37
195	MH6.Z2	SAMN10576332	SAL_ZA7698AA	Overshoes	B	Infantis	32	102	32
196	PA.329	SAMN10416368	SAL_ZA7817AA	Skin after final washing	B	Infantis	32	102	32
197	PA.340	SAMN10524035	SAL_ZA7714AA	Skin after final washing	B	Infantis	32	103	37
198	PA.341	SAMN17188253	SAL_ZA7720AA	Skin after final washing	B	Infantis	32	103	37
199	PD.330	SAMN10416395	SAL_ZA7818AA	Skin after chilling	B	Havana	588	78	25
200	PD.331	SAMN15588415	SAL_ZA7819AA	Skin after chilling	B	Havana	588	78	25
201	PD.332	SAMN15588406	SAL_ZA7820AA	Skin after chilling	B	Havana	588	78	25
202	PD.343	SAMN10524040	SAL_ZA7716AA	Skin after chilling	B	Infantis	32	103	37
203	RA1.157	SAMN20176111	SAL_ZA7727AA	Skin after final washing	B	Infantis	32	103	37
204	RA3.159	SAMN15588408	SAL_ZA7726AA	Skin after final washing	B	Infantis	32	103	37
205	RD1.160	SAMN20119332	SAL_ZA7732AA	Skin after chilling	B	Infantis	32	103	37
206	RD3.162	SAMN10741171	SAL_ZA7729AA	Skin after chilling	B	Infantis	32	102	32
207	SFA2.199	SAMN15588405	SAL_ZA7750AA	Skin after final washing	B	Infantis	32	97	1
208	Z.140	SAMN20119331	SAL_ZA7721AA	Overshoes	B	Javiana	1674	69	32
209	Z.142	SAMN11604279	SAL_ZA7722AA	Overshoes	B	Albany	292	81	24
210	Z.146	SAMN10524041	SAL_ZA7723AA	Overshoes	B	Infantis	32	102	32
211	Z.148	SAMN10524038	SAL_ZA7724AA	Overshoes	B	Infantis	32	103	37

212	Z.168	SAMN11604269	SAL_ZA7733AA	Overshoes	B	Infantis	32	103	37
213	Z.171	SAMN10741172	SAL_ZA7730AA	Overshoes	B	Infantis	32	103	37
214	Z.172	SAMN10349265	SAL_ZA7731AA	Overshoes	B	Infantis	32	101	18
215	Z.173	SAMN11604700	SAL_ZA7735AA	Overshoes	B	Infantis	32	103	37
216	Z.186	SAMN11604756	SAL_ZA7748AA	Overshoes	B	Infantis	32	103	37
217	Z.187	SAMN10397019	SAL_ZA7746AA	Overshoes	B	Infantis	32	103	37
218	Z.188	SAMN10397017	SAL_ZA7747AA	Overshoes	B	Infantis	32	101	29
219	Z.190	SAMN11604868	SAL_ZA7749AA	Overshoes	B	Liverpool	1959	76	27
220	Z.192	SAMN11604859	SAL_ZA7751AA	Overshoes	B	Infantis	32	103	37
221	ZA.271	SAMN10349325	SAL_ZA7778AA	Overshoes	B	Infantis	32	102	32
222	ZA.272	SAMN11604710	SAL_ZA7781AA	Overshoes	B	Infantis	32	103	37
223	ZA.275	SAMN11604264	SAL_ZA7780AA	Overshoes	B	Infantis	32	103	37
224	ZA.276	SAMN11640234	SAL_ZA7783AA	Overshoes	B	Infantis	32	103	37
225	ZA.277	SAMN11640645	SAL_ZA7785AA	Overshoes	B	Infantis	32	103	37
226	ZD.286	SAMN11640658	SAL_ZA7784AA	Overshoes	B	Saintpaul	50	106	2
227	ZD.287	SAMN11640636	SAL_ZA7786AA	Overshoes	B	Muenchen	83	101	10
228	ZD.288	SAMN11640683	SAL_ZA7787AA	Overshoes	B	Infantis	32	103	37
229	ZD.289	SAMN11640616	SAL_ZA7788AA	Overshoes	B	Infantis	32	103	37
230	ZD.290	SAMN11640617	SAL_ZA7789AA	Overshoes	B	Infantis	32	103	37
231	ZD.291	SAMN10397006	SAL_ZA7790AA	Overshoes	B	Infantis	32	102	21
232	ZD.292	SAMN11640657	SAL_ZA7791AA	Overshoes	B	Infantis	32	103	37
233	ZF.305	SAMN11640168	SAL_ZA7797AA	Overshoes	B	Infantis	32	103	37
234	ZF.307	SAMN10397016	SAL_ZA7798AA	Overshoes	B	Infantis	32	103	37
235	ZH.247	SAMN10741165	SAL_ZA7764AA	Overshoes	B	Infantis	32	102	32
236	ZH.248	SAMN10349288	SAL_ZA7766AA	Overshoes	B	Infantis	32	103	37
237	ZH.250	SAMN11604744	SAL_ZA7767AA	Overshoes	B	Infantis	32	103	37
238	ZH.251	SAMN11604742	SAL_ZA7769AA	Overshoes	B	Infantis	32	103	37
239	ZH.252	SAMN11604740	SAL_ZA7768AA	Overshoes	B	Infantis	32	103	37
240	ZP.333	SAMN11640153	SAL_ZA7823AA	Overshoes	B	Havana	588	78	25
241	ZP.334	SAMN10416401	SAL_ZA7821AA	Overshoes	B	Infantis	32	103	37
242	ZP.335	SAMN11640132	SAL_ZA7822AA	Overshoes	B	Infantis	32	103	37
243	ZP.337	SAMN11640227	SAL_ZA7824AA	Overshoes	B	Infantis	32	103	37

Annex 2. Assembly stats of isolates sequenced in the research.

Nº	Name	Coverage	N50	Length	Species	Contig Number (>=200 bp)	Low Quality Bases
1	O.PA115	64	24525	4801094	Salmonella enterica;99.69%	424	51746
2	O.ZC161	66	30877	4965837	Salmonella enterica;96.65%	322	43821
3	O.ZC162	255	133456	4968676	Salmonella enterica;95.23%	97	54502
4	O.ZC163	104	48755	4968484	Salmonella enterica;96.71%	204	51333
5	O.ZC164	237	80011	4980823	Salmonella enterica;94.29%	126	53050
6	O.ZC166	73	34807	4939241	Salmonella enterica;96.66%	271	56084
7	O.ZC167	175	53994	5046773	Salmonella enterica;92.36%	180	86486
8	O.ZC169	166	45613	4942554	Salmonella enterica;96.77%	211	55545
9	O.GG193	159	40643	4960850	Salmonella enterica;97.36%	253	51155
10	O.ZS214	170	70121	4973229	Salmonella enterica;96.17%	151	59411
11	O.ZS216	70	44131	4959985	Salmonella enterica;96.88%	261	50268
12	O.ZS217	84	36278	4963746	Salmonella enterica;96.73%	284	47854
13	O.ZS218	243	89010	4975218	Salmonella enterica;93.27%	138	55988
14	O.ZS222	244	81323	4960719	Salmonella enterica;94.92%	134	57686
15	O.ZS223	129	41839	4957261	Salmonella enterica;95.43%	254	58497
16	O.ZS224	98	38950	4939733	Salmonella enterica;96.44%	250	59204
17	O.ZS225	251	48383	4967440	Salmonella enterica;95.21%	218	66994
18	O.ZS226	127	78033	4941858	Salmonella enterica;96.0%	140	51438
19	O.ZS227	115	67220	4983085	Salmonella enterica;94.58%	149	34611
20	O.PCA228	163	83775	4967593	Salmonella enterica;94.92%	134	56802
21	O.PCA229	122	59487	4969433	Salmonella enterica;97.26%	163	54523
22	O.PCA230	129	53473	5040337	Salmonella enterica;96.27%	187	53490
23	O.PCA231	135	101864	4978822	Salmonella enterica;90.58%	99	54486
24	O.PCA232	172	106101	4981730	Salmonella enterica;96.59%	104	51814
25	O.PCD233	113	53679	5034411	Salmonella enterica;95.89%	189	54652
26	O.PCD234	150	62484	4967413	Salmonella enterica;94.28%	161	61609
27	O.PCD235	195	109682	4981688	Salmonella enterica;94.79%	111	55458
28	O.PCD237	113	50920	5051242	Salmonella enterica;96.04%	196	45996
29	O.ZG245	147	101835	5054068	Salmonella enterica;94.85%	113	48332
30	O.ZG246	81	47289	4991486	Salmonella enterica;99.46%	227	43927
31	O.ZG247	178	140148	4994926	Salmonella enterica;100.0%	107	48681
32	O.ZG252	109	59097	5051703	Salmonella enterica;96.04%	176	43893
33	O.ZG253	147	159252	4985029	Salmonella enterica;92.18%	78	38140
34	O.ZG254	83	70431	4952821	Salmonella enterica;97.18%	162	42327
35	O.ZG255	91	71636	4992990	Salmonella enterica;93.18%	148	43495
36	O.PSA257	87	66052	4968025	Salmonella enterica;96.4%	160	43415
37	O.PSA258	154	145856	4988901	Salmonella enterica;92.3%	92	41734
38	O.PSA259	149	94083	4987791	Salmonella enterica;94.69%	110	60966
39	O.PSA260	142	148283	4993207	Salmonella enterica;91.78%	89	31668
40	O.PSA261	137	141137	4994524	Salmonella enterica;93.06%	89	39907
41	O.CS267	152	118454	4978848	Salmonella enterica;90.8%	100	42487
42	O.PGA273	220	117132	4940384	Salmonella enterica;93.15%	85	49449
43	O.PGA274	144	102588	4937703	Salmonella enterica;94.05%	100	44286
44	O.PGA275	111	78590	4931201	Salmonella enterica;96.36%	126	48755
45	O.PGD276	142	64843	4952568	Salmonella enterica;96.4%	158	49705
46	O.PGD278	258	65190	4979872	Salmonella enterica;95.68%	160	61176
47	O.PGD280	169	149438	4980568	Salmonella enterica;93.85%	75	49438
48	O.CG281	101	36324	4938770	Salmonella enterica;95.8%	275	54914
49	O.ZP282	126	94301	4985691	Salmonella enterica;92.39%	114	37242
50	O.ZP287	171	95459	4990874	Salmonella enterica;92.3%	105	51555
51	O.ZP288	133	93555	4965417	Salmonella enterica;94.69%	131	56646
52	O.ZP290	103	50044	4958850	Salmonella enterica;95.73%	214	51971
53	O.PNA294	159	85327	4944159	Salmonella enterica;96.2%	123	78351
54	O.PNA295	109	107452	4952496	Salmonella enterica;95.46%	104	33445
55	O.PNA296	163	145922	5064987	Salmonella enterica;90.54%	86	59888
56	O.PNA297	207	75575	4936498	Salmonella enterica;96.92%	150	63663
57	O.PNA298	144	86535	4981213	Salmonella enterica;94.83%	131	43958
58	O.PND299	139	81697	5030557	Salmonella enterica;91.74%	143	61823
59	O.PND300	170	93849	4972106	Salmonella enterica;96.27%	125	38324
60	O.PND301	238	89312	4978113	Salmonella enterica;93.52%	127	62613
61	O.PND302	165	64105	4974066	Salmonella enterica;95.62%	155	38535
62	O.PND303	156	49379	4979781	Salmonella enterica;96.85%	228	41225
63	O.PPA305	198	112822	4979462	Salmonella enterica;93.76%	110	39503
64	O.PPA306	163	118637	4982244	Salmonella enterica;93.18%	99	44772
65	O.PPA308	135	114498	4979454	Salmonella enterica;92.94%	99	38973
66	O.PPA309	121	61614	4999468	Salmonella enterica;96.0%	145	69498
67	O.PPD312	194	86197	4979658	Salmonella enterica;93.8%	126	63077
68	O.PPD313	183	152360	4985987	Salmonella enterica;94.6%	89	42994
69	2CTA.022	246	65398	4950424	Salmonella enterica;99.24%	168	37897
70	2CTA.040	231	197812	4647189	Salmonella enterica;100.0%	59	30140
71	2CTA.056	231	134540	4730661	Salmonella enterica;100.0%	97	41306
72	2CTA.058	190	45702	4589158	Salmonella enterica;100.0%	189	62664
73	2CTA.088	227	76691	4735039	Salmonella enterica;96.36%	140	37508
74	2CTA.119	188	71917	4756844	Salmonella enterica;98.43%	159	68739
75	2CTA.159	215	76690	4716732	Salmonella enterica;98.14%	133	55889
76	2CTA.170	148	62085	4719400	Salmonella enterica;98.34%	150	56774
77	2CTP.0017	203	56760	5046971	Salmonella enterica;99.33%	177	73466
78	2CTP.022	236	148432	4645337	Salmonella enterica;96.36%	66	58151
79	AA.309	233	147425	4967164	Salmonella enterica;97.22%	86	51704

80	AA.310	199	52159	4947876	Salmonella enterica;97.76%	210	63264
81	AA.311	174	51442	4956842	Salmonella enterica;96.74%	211	59903
82	AD.312	128	50029	4958864	Salmonella enterica;96.28%	223	42094
83	AD.313	90	57654	4967397	Salmonella enterica;94.97%	178	55285
84	AD.314	235	31888	5076591	Salmonella enterica;93.64%	364	83856
85	BA.315	214	184340	4985093	Salmonella enterica;91.23%	72	41115
86	BA.316	151	46670	4975475	Salmonella enterica;95.55%	234	49275
87	BA.317	239	145856	4986440	Salmonella enterica;92.75%	86	50189
88	BD.318	116	42982	5091581	Salmonella enterica;95.48%	264	66218
89	BD.319	168	106101	5094246	Salmonella enterica;93.5%	92	53461
90	BD.320	172	53255	4978932	Salmonella enterica;96.55%	204	49802
91	CA2.205	151	126735	4961653	Salmonella enterica;91.95%	104	52345
92	DA.294	297	97045	4965941	Salmonella enterica;96.74%	107	60980
93	DA.295	259	145757	4970748	Salmonella enterica;96.22%	93	63677
94	DA.296	289	109422	4969571	Salmonella enterica;96.39%	95	65348
95	DD.299	96	52218	4734070	Salmonella enterica;100.0%	167	33323
96	FA.321	269	126037	4937127	Salmonella enterica;92.94%	99	51343
97	FA.322	136	55956	4972827	Salmonella enterica;95.27%	171	50423
98	FA.323	132	56205	4969579	Salmonella enterica;96.03%	185	39123
99	FD.324	158	48074	4959446	Salmonella enterica;95.63%	241	60037
100	FD.325	160	184346	4983192	Salmonella enterica;88.33%	73	40364
101	FD.326	100	98469	4969635	Salmonella enterica;91.76%	113	40925
102	HA1.210	159	53459	4959328	Salmonella enterica;95.81%	192	48906
103	HA1.265	176	119140	4962203	Salmonella enterica;97.45%	95	58037
104	HA2.211	148	47056	4957367	Salmonella enterica;95.99%	237	54418
105	HA2.266	194	79999	4959048	Salmonella enterica;98.52%	158	50964
106	HA3.212	110	23137	4900215	Salmonella enterica;96.87%	419	69125
107	HA3.267	50	159250	5132096	Salmonella enterica;73.44%	73	22789
108	HC.086	216	135699	4944694	Salmonella enterica;97.73%	110	56302
109	HC.087	178	131368	4949892	Salmonella enterica;98.99%	125	46478
110	HC.106	246	80245	4948573	Salmonella enterica;98.12%	139	35034
111	HC.111	186	140291	4694570	Salmonella enterica;97.02%	86	53311
112	HC.128	158	131298	4771029	Salmonella enterica;98.68%	103	28735
113	HC.130	123	68445	4940998	Salmonella enterica;98.49%	161	44592
114	HC.132	165	44740	4944138	Salmonella enterica;98.53%	254	45708
115	HC.155	240	189786	4684358	Salmonella enterica;100.0%	84	57439
116	HC.219	95	33813	4952515	Salmonella enterica;99.59%	315	50853
117	HC.220	188	49729	4940060	Salmonella enterica;98.37%	216	55882
118	HC.227	158	49155	4592601	Salmonella enterica;100.0%	182	41612
119	HC.228	183	65549	4977050	Salmonella enterica;98.57%	173	48370
120	HC.232	134	72667	4942530	Salmonella enterica;99.1%	140	49745
121	HC.238	54	27184	4568369	Salmonella enterica;100.0%	328	48181
122	HC.245	116	103210	4951737	Salmonella enterica;97.84%	122	24870
123	HC.246	122	93313	4949599	Salmonella enterica;99.0%	124	42561
124	HC.255	419	309563	4953960	Salmonella enterica;100.0%	62	27326
125	HC.257	185	204015	4644478	Salmonella enterica;95.24%	60	25290
126	HC.264	174	52713	4728869	Salmonella enterica;98.71%	190	66477
127	HC.285	116	58872	4972158	Salmonella enterica;99.36%	191	50972
128	HC.304	137	49718	4939448	Salmonella enterica;98.91%	218	51132
129	HD1.213	143	39158	4962760	Salmonella enterica;96.87%	226	48349
130	HD1.268	244	49972	4971755	Salmonella enterica;94.74%	195	48426
131	HD2.269	140	152360	4939517	Salmonella enterica;91.42%	84	37099
132	HD3.270	144	141180	4951116	Salmonella enterica;91.25%	95	48383
133	HS.126	192	113156	4977896	Salmonella enterica;98.93%	113	43303
134	HS.133	220	36328	4965316	Salmonella enterica;99.13%	286	57147
135	HS.260	179	127455	4980572	Salmonella enterica;100.0%	106	40856
136	IA1.174	129	68965	4972972	Salmonella enterica;95.45%	176	48669
137	IA2.175	153	47023	4952646	Salmonella enterica;95.05%	203	72453
138	IA3.176	168	39831	4962492	Salmonella enterica;95.83%	248	55517
139	ID1.177	232	180709	4971047	Salmonella enterica;91.8%	78	44926
140	ID2.178	209	162691	4987699	Salmonella enterica;92.07%	79	38069
141	MA1.180	295	83773	4980187	Salmonella enterica;90.65%	124	58981
142	MA2.181	158	64967	4975343	Salmonella enterica;95.17%	166	47078
143	MA3.182	146	45517	4973202	Salmonella enterica;95.12%	240	47842
144	MD1.183	155	191935	4982808	Salmonella enterica;94.83%	73	34228
145	MD2.184	98	29683	4946590	Salmonella enterica;96.27%	315	59860
146	MD3.185	174	45737	4969874	Salmonella enterica;97.32%	220	58484
147	MH1.A1	191	74471	4973279	Salmonella enterica;96.85%	145	58653
148	MH1.A4	132	97045	4980184	Salmonella enterica;94.69%	111	49133
149	MH1.D4	161	101660	4972076	Salmonella enterica;95.05%	119	54915
150	MH2.A1	198	119704	4993421	Salmonella enterica;95.41%	105	49368
151	MH2.A3	272	98534	4992374	Salmonella enterica;92.93%	116	59079
152	MH2.D1	193	97470	4992657	Salmonella enterica;93.54%	109	58890
153	MH2.D4	197	159250	4994986	Salmonella enterica;92.41%	92	49385
154	MH2.Z1	215	124923	5002119	Salmonella enterica;89.87%	95	57338
155	MH3.A1	108	72722	4986622	Salmonella enterica;96.12%	151	41923
156	MH3.A2	151	154458	4985780	Salmonella enterica;94.94%	96	37109
157	MH3.A3	228	90348	4980588	Salmonella enterica;96.37%	126	60352
158	MH3.C	114	184340	4994730	Salmonella enterica;92.96%	90	28642
159	MH3.D1	235	86265	4978602	Salmonella enterica;97.36%	135	57516
160	MH3.D2	113	64573	4983193	Salmonella enterica;96.51%	161	54249
161	MH3.D3	220	91543	4978992	Salmonella enterica;96.15%	120	64768
162	MH3.D4	189	69155	4974028	Salmonella enterica;95.68%	156	91302
163	MH3.Z1	102	53132	4976556	Salmonella enterica;96.92%	182	37522
164	MH3.Z2	183	107168	4671376	Salmonella enterica;100.0%	88	50303
165	MH4.A1	221	181177	4988718	Salmonella enterica;94.45%	65	26311

166	MH4.A2	239	136666	4981357	Salmonella enterica;96.11%	93	56131
167	MH4.A3	289	77355	4979330	Salmonella enterica;96.67%	137	62547
168	MH4.A4	123	204015	4991821	Salmonella enterica;89.59%	65	12259
169	MH4.D1	149	53916	4974201	Salmonella enterica;96.3%	181	52986
170	MH4.D2	202	194249	5041959	Salmonella enterica;91.24%	78	78136
171	MH4.D3	307	76543	4974540	Salmonella enterica;94.3%	144	59850
172	MH4.D4	147	122767	5038457	Salmonella enterica;91.25%	104	81643
173	MH4.Z2	58	180546	4988669	Salmonella enterica;88.52%	80	21576
174	MH5.A1	126	62095	4973036	Salmonella enterica;97.19%	205	43726
175	MH5.A2	173	92098	4986109	Salmonella enterica;95.05%	119	76180
176	MH5.A3	144	55612	4976491	Salmonella enterica;95.45%	184	41320
177	MH5.A4	155	55869	4971242	Salmonella enterica;97.54%	187	58130
178	MH5.C	149	76972	4979358	Salmonella enterica;96.44%	130	40345
179	MH5.D1	133	45170	4972145	Salmonella enterica;97.74%	253	71216
180	MH5.D2	192	76972	4991814	Salmonella enterica;93.89%	149	77138
181	MH5.D3	189	97038	4991744	Salmonella enterica;93.94%	116	65951
182	MH5.D4	148	73465	4982604	Salmonella enterica;96.72%	144	48621
183	MH5.Z1	141	58711	4981083	Salmonella enterica;95.1%	164	38619
184	MH5.Z2	121	98075	4979192	Salmonella enterica;96.0%	118	35100
185	MH6.A1	267	204015	4980272	Salmonella enterica;93.62%	58	36849
186	MH6.A2	213	185578	4987470	Salmonella enterica;94.74%	69	35870
187	MH6.A3	311	184346	4987198	Salmonella enterica;94.22%	65	38280
188	MH6.A4	162	93322	4971567	Salmonella enterica;95.15%	118	57588
189	MH6.C	195	72233	4965121	Salmonella enterica;96.33%	156	58650
190	MH6.D1	206	86423	4978798	Salmonella enterica;95.28%	122	51310
191	MH6.D2	198	90515	4976995	Salmonella enterica;98.0%	114	57101
192	MH6.D3	152	96117	4976533	Salmonella enterica;97.83%	109	43392
193	MH6.D4	129	51653	5035659	Salmonella enterica;95.34%	187	72245
194	MH6.Z1	185	131623	4987233	Salmonella enterica;92.94%	103	43718
195	MH6.Z2	136	59090	5035023	Salmonella enterica;97.52%	186	59546
196	PA.329	140	53777	4977471	Salmonella enterica;96.97%	190	45157
197	PA.340	70	80209	4985190	Salmonella enterica;95.28%	142	26506
198	PA.341	357	180709	4969353	Salmonella enterica;87.93%	75	34797
199	PD.330	183	55009	4929036	Salmonella enterica;95.51%	188	45766
200	PD.331	136	200482	4923441	Salmonella enterica;94.28%	84	51795
201	PD.332	121	141021	4920761	Salmonella enterica;94.66%	91	39419
202	PD.343	80	98545	5094173	Salmonella enterica;93.75%	114	25999
203	RA1.157	323	41771	4970170	Salmonella enterica;96.63%	249	58245
204	RA3.159	202	118732	4983078	Salmonella enterica;92.5%	96	53479
205	RD1.160	297	61919	5022035	Salmonella enterica;95.4%	179	119896
206	RD3.162	81	42641	4981873	Salmonella enterica;96.11%	242	41207
207	SFA2.199	90	25460	4891209	Salmonella enterica;97.12%	348	104512
208	Z.140	230	106871	4501361	Salmonella enterica;100.0%	82	36386
209	Z.142	159	351007	4784053	Salmonella enterica;100.0%	43	27730
210	Z.146	160	96908	4991932	Salmonella enterica;89.25%	112	32092
211	Z.148	143	106920	4984985	Salmonella enterica;92.59%	99	37368
212	Z.168	258	92409	4975568	Salmonella enterica;95.75%	111	67828
213	Z.171	89	50374	4964217	Salmonella enterica;96.67%	208	44665
214	Z.172	55	34196	4959075	Salmonella enterica;97.72%	316	51351
215	Z.173	255	184340	4984720	Salmonella enterica;93.23%	75	35697
216	Z.186	234	49838	4979108	Salmonella enterica;96.69%	213	62272
217	Z.187	161	50920	4980488	Salmonella enterica;96.38%	190	45510
218	Z.188	85	32886	4966183	Salmonella enterica;97.33%	308	36809
219	Z.190	182	248170	4740180	Salmonella enterica;96.97%	55	47205
220	Z.192	177	245776	4987557	Salmonella enterica;93.62%	63	20343
221	ZA.271	129	56134	4958014	Salmonella enterica;96.27%	181	43848
222	ZA.272	225	62456	4939423	Salmonella enterica;95.1%	159	76893
223	ZA.275	137	204015	4971304	Salmonella enterica;92.72%	69	23657
224	ZA.276	219	145757	4966537	Salmonella enterica;94.02%	82	59136
225	ZA.277	230	194394	4915956	Salmonella enterica;90.74%	69	44815
226	ZD.286	102	255937	4873423	Salmonella enterica;93.88%	67	23009
227	ZD.287	175	270824	4620166	Salmonella enterica;90.63%	47	55645
228	ZD.288	184	60717	4976395	Salmonella enterica;96.53%	161	100949
229	ZD.289	238	114067	4978868	Salmonella enterica;95.45%	105	65368
230	ZD.290	182	180709	4984766	Salmonella enterica;92.85%	68	61156
231	ZD.291	161	58872	4978735	Salmonella enterica;96.8%	182	38344
232	ZD.292	201	180709	4983923	Salmonella enterica;92.45%	70	59790
233	ZF.305	217	148754	4966707	Salmonella enterica;91.95%	76	64159
234	ZF.307	139	64133	4946675	Salmonella enterica;95.75%	164	36362
235	ZH.247	72	148283	5046084	Salmonella enterica;93.9%	97	28362
236	ZH.248	103	145922	4972460	Salmonella enterica;93.05%	90	17609
237	ZH.250	156	167173	4961030	Salmonella enterica;92.98%	71	35715
238	ZH.251	269	70739	4954048	Salmonella enterica;95.07%	139	78053
239	ZH.252	156	118715	5059904	Salmonella enterica;90.69%	99	70504
240	ZP.333	224	143361	4941126	Salmonella enterica;98.57%	86	64101
241	ZP.334	163	70743	4974116	Salmonella enterica;95.31%	148	60797
242	ZP.335	153	245776	4988792	Salmonella enterica;89.8%	64	26783
243	ZP.337	169	194246	4969702	Salmonella enterica;92.45%	65	45503