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Contribution to the knowledge of the physiology, involved foods, and control of nontyphoidal *Salmonella enterica*

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Contribution to the knowledge of the physiology, involved foods, and control of nontyphoidal *Salmonella enterica*

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RESUMEN

A nivel mundial, *Salmonella* no tifoidea (SNT) es la principal causa de enfermedades bacterianas transmitidas por los alimentos. SNT ha sido aislada de una gran variedad de productos, pero más frecuentemente, de alimentos de origen animal; sin embargo, su creciente presencia en frutas y vegetales también constituye un riesgo de salmonelosis porque estos alimentos suelen consumirse crudos. Aunque la salmonelosis de origen alimentario es generalmente leve, se estima que la carga económica global asociada a salmonelosis humana es mayor a 2600 millones de dólares anuales; por esta razón, las normas sanitarias son muy estrictas en cuanto a la baja o nula tolerancia de SNT en alimentos. El incumplimiento de las normas en cuanto al control de este patógeno en la industria de alimentos ocasiona pérdidas económicas importantes para toda la cadena productiva. A este panorama hay que añadir la preocupación mundial por el incremento de la resistencia antimicrobiana (RAM) en bacterias, en vista de que hay numerosos reportes de SNT que presentan resistencia a varios antimicrobianos. En este contexto, esta investigación tuvo como componentes: (1) una revisión sistemática de publicaciones sobre alimentos en los que se ha reportado SNT, sus serotipos y patrones de RAM en América Latina; (2) la determinación de la capacidad de *Salmonella* de crecer en materia fecal fresca de pollo, con el fin de conocer su comportamiento una vez que es excretada de un hospedador animal, lo que puede repercutir en la contaminación de alimentos; y (3) el aislamiento de mezclas de bacteriófagos de aguas de desecho, y su aplicación en una granja avícola comercial, como parte de una estrategia de búsqueda de sustitutos de los antibióticos. De este trabajo se puede concluir que, con el aumento de la producción de alimentos a gran escala, y por la capacidad que tiene SNT de multiplicarse en la materia fecal fuera del hospedador, el uso de estiércol como fertilizante sin un tratamiento adecuado contribuye a que este patógeno contamine productos agrícolas; ante este riesgo son, necesarias

acciones de control apropiadas para evitar su diseminación e ingreso a la cadena alimenticia. Por otro lado, con los resultados obtenidos se puede afirmar que los bacteriófagos constituyen una alternativa viable para reemplazar a los antibióticos contra *Salmonella* en la industria avícola; sin embargo, a futuro es necesario monitorear la propagación de bacterias resistentes a los bacteriófagos.

Palabras clave: *Salmonella* no tifoidea, alimentos, América Latina, materia faecal de pollo, bacteriófagos de *Salmonella*, granjas avícolas.

ABSTRACT

Globally, nontyphoidal *Salmonella* (NTS) is the leading cause of foodborne bacterial diseases; it has been isolated from a wide variety of products, but more frequently from foods of animal origin; however, its increasing presence in fruits and vegetables also constitutes a risk of salmonellosis because these foods are usually eaten raw. While foodborne salmonellosis is generally mild, the global economic burden associated with human salmonellosis is estimated to be greater than 2.6 billion dollars annually; for this reason, sanitary standards are very strict regarding the low or null tolerance of NTS in food. The non-observance of the regulations in the control of this pathogen in the food industry gives rise to significant economic losses for the entire production chain. To this panorama must be added the worldwide concern about the increase in antimicrobial resistance (AMR) in bacteria, given that there are numerous reports of multidrug-resistant NTS. In this context, this research had as components: (1) A systematic review of publications about foods in which NTS, serotypes and AMR patterns have been reported in Latin America; (2) the determination of the ability of *Salmonella* to grow in fresh chicken faecal matter, to know its behavior once it is excreted from an animal host, which may have repercussions on food contamination; and (3) the isolation of bacteriophages from wastewater, and their application on a commercial poultry farm as part of a search strategy for antibiotic substitutes. From this work it can be concluded that, with the increase in large-scale food production, and due to the NTS ability to multiply in faeces outside the host, the use of manure as fertilizer without adequate treatment contributes to this pathogen contaminating produce. Given this risk, appropriate control actions are necessary to prevent NTS dissemination and entry into the food chain. On the other hand, based on the results obtained, it can be affirmed that bacteriophages constitute a viable alternative to replace antibiotics

against *Salmonella* in the poultry industry; however, it is necessary to monitor the spread of bacteriophage-resistant bacteria in the future.

Keywords: Nontyphoidal *Salmonella*, food, Latin America, chicken faecal matter, *Salmonella* bacteriophages, poultry farms.

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CHAPTER 1

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Systematic review: Non-typhoidal *Salmonella* in food from Latin America

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Abstract

In Latin America, nontyphoidal *Salmonella* (NTS) is one of the most important etiological agents of foodborne infections; it can survive in soil, water, and food even after processing. Here, we aimed to perform a systematic review by collecting data on the prevalence, serotypes, and antimicrobial resistance (AMR) of NTS isolated from different food products in Latin America, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Out of 1766 studies screened, 244 reports from 13 Latin American countries were eligible. Among these, 182 reported NTS prevalence, 87 reported NTS serotypes, and 83 reported serotypes with AMR patterns. The NTS prevalence ranged from 0.005% to 93.3%, regardless of country and food. Meat showed the highest NTS prevalence. Enteritidis, Typhimurium, and Derby were the most frequently observed serotypes in different food products. The serotypes Enteritidis, Typhimurium, and Infantis, isolated from animal products,

showed the highest AMR rate. The presence of NTS in fruits and vegetables, which are generally consumed raw or as ready-to-eat food, indicates a high risk of salmonellosis from consuming these foods. Thus, the reduction of this pathogen in the food chain requires a One Health approach, involving good agricultural and manufacturing practices, low antimicrobial use, and proper waste management.

Keywords: Non-typhoidal *Salmonella*, food, Latin America

Introduction

According to the Food and Agricultural Organization of the United Nations (FAO), Latin America produces enough food to feed its population (FAO, 2020a); this has resulted in the intensification of agriculture and commercial trade with consequential environmental deterioration (Kopittke *et al.*, 2019; Kaimowitz, 2020). These conditions increase the prevalence of zoonotic foodborne pathogens in farm animals and crops (FAO, 2020a).

In low- and middle-income countries, including most Latin America countries, the use of wastewater for agricultural purposes and animal waste as fertilizers is not regulated (Khalid *et al.*, 2018), which can lead to pathogen contamination of edible crops (Mandrell, 2009).

In Latin America, ~77 million people become ill and 9000 die annually owing to contaminated food. *Norovirus*, *Campylobacter*, *Escherichia coli*, and nontyphoidal *Salmonella* (NTS) account for 95% of food poisoning cases in the Region of the Americas (WHO, 2015). Foodborne illnesses result in financial losses for governments, producers, and consumers (FAO, 2020b). In addition, Latin America lacks funding for food safety research (Jaffee *et al.*, 2019). These data highlight the need for improvement of national public policies regarding agriculture and food safety, as well as regional programs for the prevention, control, and eradication of pests and animal diseases (FAO, 2020a).

Salmonella is a ubiquitous bacterium that can survive for several weeks in soil, water, and food (WHO, 2018); it can also survive and multiply in fresh faecal matter (Guerrero et al., 2020). *Salmonella* can also grow on plants; therefore, fruits and vegetables may act as routes of *Salmonella* entry into the food chain (Silva et al., 2014). In animal products, NTS infection occurs through feed or environmental contamination (Hoelzer et al., 2011; WHO, 2018).

NTS is the leading bacterial cause of foodborne illnesses worldwide (CDC, 2020). Most nontyphoidal salmonellosis cases are mild; however, they may lead to life-threatening complications in children, the elderly, and people with disabilities. NTS infection severity depends on host immunity, *Salmonella* serotype, and infective dose. In addition, antimicrobial-resistant NTS serotypes have emerged, contributing to the global public health crisis (WHO, 2018), including NTS serotypes that are multidrug-resistant (MDR; resistant to ≥ 3 antimicrobial classes), third-generation cephalosporin resistant (3GCr), fluoroquinolone nonsusceptible (FQNS), and extended-spectrum beta-lactamase (ESBL) producers (WHO, 2017; McDermott et al., 2018; Tack et al., 2020).

Here, we aimed to perform a systematic review of publications selected based on defined inclusion criteria for the detection and prevalence of NTS serotypes in food from Latin American countries, as well as the evaluation of antimicrobial resistance (AMR) patterns of these serotypes.

Methods

Research strategy

We used the following inclusion criteria: 1) articles published in English, Portuguese, and Spanish; 2) primary research reports; 3) NTS isolated from food; and 4) food originating in Latin American countries (Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Mexico, Nicaragua,

Panama, Paraguay, Peru, Puerto Rico, Uruguay, or Venezuela). A systematic review was carried out in the following four steps: 1) bibliographic source identification, 2) record screening, 3) record eligibility, and 4) record inclusion (Moher *et al.*, 2015; Cardona-Arias *et al.*, 2016) (Fig. 1).

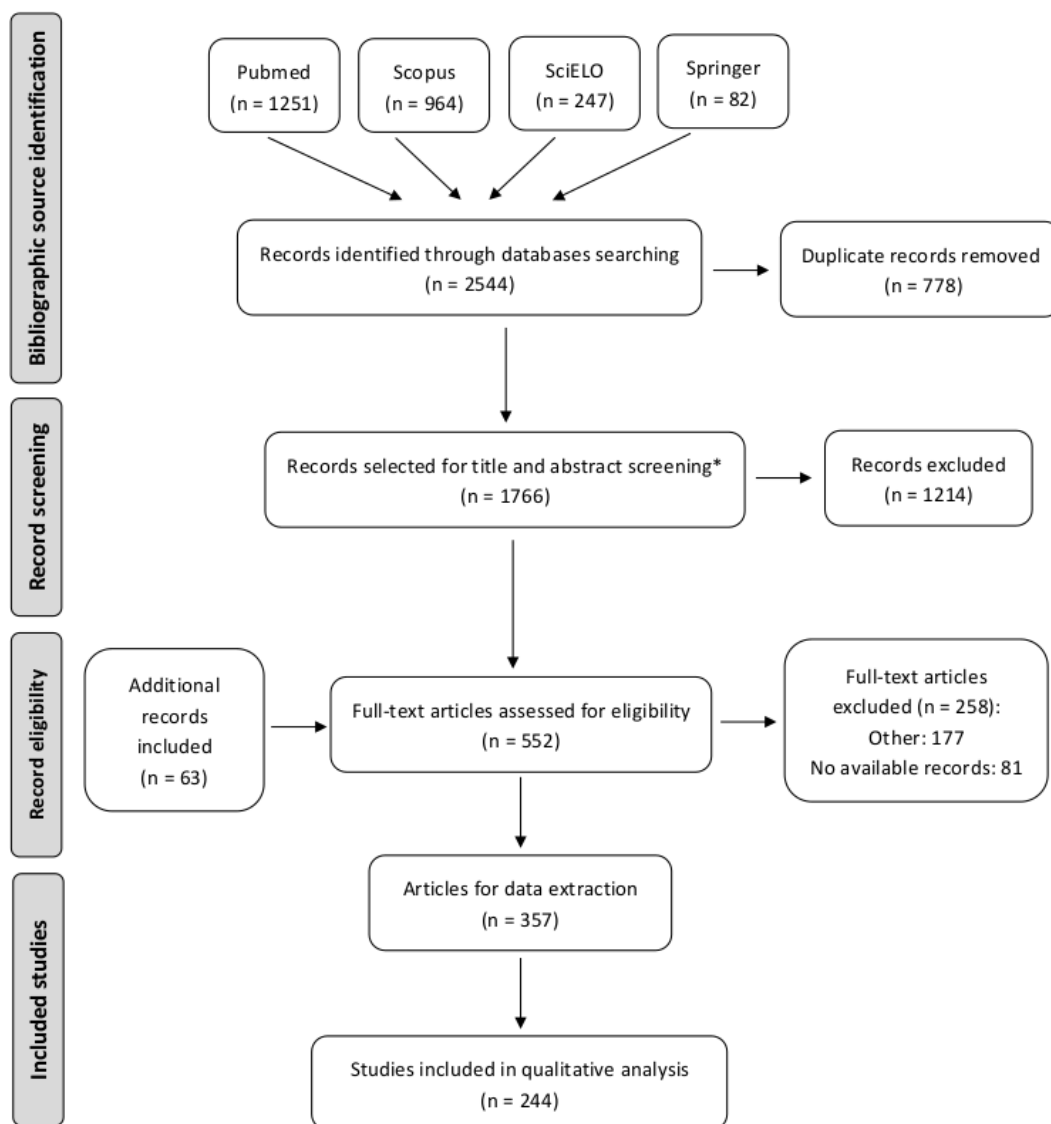


FIG. 1. PRISMA flow diagram of the literature search and data extraction. From Moher *et al.* (2009).

Bibliographic source identification

The electronic databases used were PubMed, Scopus, SciELO, and Springer. The following search terms were used: "*Salmonella*" AND ([food OR bread OR cooking OR drink OR feed OR foodstuff OR meal OR meat OR snack] AND [Argentina OR Bolivia OR Brazil OR Chile OR Colombia OR "Costa Rica" OR Cuba OR "Dominican Republic" OR Ecuador OR "El Salvador" OR Guatemala OR Haiti OR Honduras OR Mexico OR Nicaragua OR Panama OR Paraguay OR Peru OR "Puerto Rico" OR Uruguay OR Venezuela]). The last research data were obtained on July 19, 2020. No publication date restrictions were applied (Supplementary Table S1).

Record screening

In the first screening, T.G. removed duplicate records. To minimize bias, T.G. and R.B.-R. selected articles based on titles and abstracts according to the inclusion criteria. A third reviewer (S.Z.M.) resolved disagreements between the first two reviewers.

Record eligibility

The last screening and data extraction of all selected publications were performed by T.G., R.B.-R., and E.E. The following information was extracted: 1) country from which the food originated, 2) sampling date, 3) analyzed food, 4) NTS serotypes, 5) laboratory methods, 6) sample size, 7) prevalence, and 8) AMR of the isolates. We excluded studies on NTS from nonfood sources, that did not identify NTS using laboratory methods, that did not specify the food source of NTS, and in which the food's origin was not Latin American. The final review of the results and the approval of the article were carried out by S.Z.M.

Record inclusion

The qualitative analysis included previously chosen publications containing information on NTS prevalence, serotypes, and AMR and additional articles obtained through manual screening of the reference lists of the retrieved reviews, systematic reviews, and meta-analyses. For AMR reports we used four criteria: MDR, 3GCr, FQNS, and ESBL producers. In this

review, we reported the prevalence as described in the original studies, and the sample size, represented by n .

Results

Search results and study selection

We obtained 2544 records from the databases, which included 778 duplicate records. Ultimately, 1766 original records were available for further screening. After selection by titles and abstracts, 552 records were assessed for eligibility. Of these, 258 were excluded: 177 were removed based on the inclusion criteria, and 81 were removed owing to unavailability of full texts in the databases. We also included 63 records extracted from reviews, systematic reviews, and meta-analyses. Finally, we had a total of 357 records for data extraction; of these, 244 were included in the qualitative analysis (Fig. 1). These records were reported from 13 Latin American countries (Fig. 2).



FIG. 2. Number of articles from which the data were extracted, by country.

We classified the selected records based on country, NTS prevalence, serotypes, and AMR patterns. A total of 182 records from 13 countries reported NTS prevalence, 87 from 12 countries reported *Salmonella* serotypes, and 83 from 10 countries reported serotypes with AMR patterns (Fig. 2). In addition, we grouped the food into meat (chicken, bovine meat, pork), vegetables, fruits, dairy products, sausages, eggs, seafood, spices, animal feed, ready-to-eat (RTE), and other foods.

Reported prevalence of NTS in various food products

Mexico and Brazil had the largest number of food groups analyzed. The NTS prevalence ranged from 0.005% to 93.3%, regardless of country and food (Table 1). The highest NTS prevalence

was found in chicken, pork and bovine meat from Brazil, Colombia, Ecuador, Mexico, and Venezuela; the lowest prevalence was found in eggs from Chile and Uruguay. In particular, Venezuela, Ecuador and Colombia reported the highest NTS prevalence in chicken, whereas Brazil and Mexico in pork, and Ecuador and Brazil in bovine meat (60%–93.3%). In contrast, Paraguay showed the lowest prevalence in chicken, Argentina and Peru in pork, and Uruguay and Costa Rica in bovine meat (0.4%–7%).

A wide range of NTS prevalence (4.4%–88.3%) was found in sausages, with Mexican pork sausage having the highest contamination prevalence ($n=200$). In dairy products, the prevalence range was 0.4%–62.9%, with Colombian fresh cheese being the most frequently contaminated product ($n=27$). Vegetables, fruits, and their derivatives presented a prevalence range of 0.4%–40%. Farmed fish and seafood products showed a considerable range of NTS prevalence of 0.9%–50%, with Peruvian shrimp being the most contaminated ($n=2$).

The NTS prevalence range in animal feed was 1.3%–66.7% and that in animal feed raw materials was 0.5%–26.7%. Chocolate, peanuts, and RTE food showed the lowest contamination (< 5%).

Distribution and diversity of NTS serotypes in various food products

Overall, a total of 39 serotypes were reported in studies from the selected countries, with Brazil (22), Mexico (11), and Colombia (11) having the most available data. Some serotypes were present in only one country, such as Brazil (12), Colombia (6), Mexico (3), Costa Rica (1), Cuba (1), and Venezuela (1) (Table 2 Supplementary Table S2). The greatest diversity of serotypes in a wide variety of foods was detected in Brazil.

Enteritidis, Typhimurium, and Derby were the most frequent *Salmonella* serotypes in different food products of several countries. Here, Enteritidis was found mainly in chicken products, although it was also reported in vegetables, processed food, and chili sauce. Typhimurium was

present in both plant and animal products. Derby was found in pork products, chocolate, eggs, and RTE food.

The serotypes Infantis and Enteritidis were reported frequently in Chile and Ecuador, mostly in chicken products. Infantis was also reported in Brazilian pork and Cuban dairy products. The serotype Heidelberg was found at a low rate (< 40%) in chicken products from Brazil and Venezuela but predominated the feedstuff from Costa Rica (Fig. 3). Uganda was the most frequent serotype in Colombian meat, but it was not found in food products from other countries. The serotypes Rubislaw and Miami were reported only in drinking water and peanuts in Colombia and Brazil, respectively.



FIG. 3. Main nontyphoidal *Salmonella* serotypes and related food, by country.

The most common serotypes isolated from chicken were Enteritidis, Infantis, Albany, and Schwarzengrund in Chile, Brazil, and Ecuador; chicken giblets presented the serotypes Infantis, Typhimurium, Enteritidis, and Schwarzengrund in Ecuador, Mexico, Chile, and Argentina. Enteritidis and Senftenberg were found in eggs in Chile, Ecuador, Colombia, and Brazil. In

bovine meat, Newport and Livingstone were reported in Uruguay and Brazil, whereas in pork, Typhimurium, Derby, and Infantis were reported in Argentina, Peru, and Brazil (Fig. 3). A high frequency of Typhimurium (47%–100%) and a low frequency of the serotypes Bredeney, Derby, Anatum, and Saintpaul (< 34%) were found in Brazilian pork sausages. In Mexico, the serotypes Typhimurium and Enteritidis were detected in vegetables, and Typhimurium and Agona were found in fruits. Typhimurium, Enteritidis, Agona, and Anatum were found in Mexican fruit juices, and Choleraesuis was reported in Brazilian orange juice. In Brazilian seafood, the frequency of the serotypes Senftenberg and Panama was high (> 70%) whereas that of Tennessee and Poona was low (< 28%). Finally, the serotypes Havana, Heidelberg, and Infantis were common in animal feed from Costa Rica and Ecuador, whereas Senftenberg was detected in low percentages in animal feed from Brazil.

AMR of NTS in various food products

We found 83 publications with AMR patterns of more than 5500 NTS isolates belonging to 75 serotypes. For the AMR analysis, we only considered 66 publications containing the sampling date of the tested food (from 1975 to 2017) (Supplementary Table S3).

The most frequent resistance was to tetracycline, ampicillin, streptomycin, nalidixic acid, sulfamethoxazole-trimethoprim, chloramphenicol, and gentamicin. Resistance to tetracycline, ampicillin, streptomycin, and gentamicin has been reported since 1975, whereas that to nalidixic acid, sulfamethoxazole-trimethoprim, and chloramphenicol has only been reported since 1995. The first reports of 3GCr (cefotaxime and ceftriaxone) and FQNS (norfloxacin) were for isolates from poultry meat and giblets in Brazil in 1995.

The most common antimicrobial-resistant NTS serotypes were Typhimurium, Enteritidis and Infantis. MDR-3GCr Typhimurium strains were found in pork and poultry from Brazil, Colombia, and Mexico, whereas MDR-FQNS and MDR-3GCr-FQNS strains were predominantly in pork from Brazil, Ecuador, and Mexico. MDR, MDR-FQNS, and MDR-

3GCr-FQNS Enteritidis isolates were found in poultry, eggs, egg-meals, and poultry products from Argentina, Brazil, and Venezuela. MDR Infantis strains were isolated from pork, poultry, and beef; MDR-3GCr, MDR-FQNS, and MDR-3GCr-FQNS strains were present in pork and poultry from Brazil, Colombia, Ecuador, and Mexico. The most frequent ESBL producers were Heidelberg and Typhimurium, isolated from chicken and turkey in Brazil and Venezuela, with the first report from the year 2000.

Discussion

Foodborne diseases caused by NTS are a global public concern, which becomes more acute with the increase in AMR among foodborne NTS isolates. Our findings reveal important data on the prevalence of NTS and the diversity of antimicrobial-resistant NTS serotypes.

NTS prevalence was over 70% in some raw animal products (pork, beef, and chicken). This is comparable with that reported by Contreras-Soto *et al.*, thus suggesting that, although *Salmonella* can survive in soil and water, most food contamination with NTS originates from animal sources. Healthy animals can carry NTS, and their carcasses become contaminated with gastrointestinal content during slaughter (Gómez-Aldapa *et al.*, 2012; Contreras-Soto *et al.*, 2019). In Mexico, the skin and lymph nodes have been identified as potential sources of NTS contamination of bovine meat (Gragg *et al.*, 2013), whereas in Brazil, the presence of NTS in pork has been associated with residual environmental contamination and contaminated feed (Tondo and Ritter, 2012).

Most dairy-borne *Salmonella* infections have been associated with raw or improperly pasteurized milk or post-pasteurization contamination (El-Gazzar and Marth, 1992). We identified fresh Colombian cheese as a source of *Salmonella*, suggesting deficiencies in processing, transportation, storage, and/or retail marketing (de Souza *et al.*, 2017).

The low prevalence of NTS among eggs in Uruguay could be due to factors, such as vaccination against *Salmonella* in laying hens, type of housing system, cleaning and disinfection protocols in farms, water sanitation, and feed safety. Anti-NTS vaccination is a control measure, but its effectiveness decreases if bird environments contain high bacterial loads (Betancor *et al.*, 2010; Terzolo, 2010).

NTS in edible vegetables, such as Brazilian collard greens or Mexican melons, has been associated with the use of manure (mainly from pigs or poultry) for fertilization and contaminated water for irrigation, and the lack of hygiene protocols for workers handling products (ICMSF, 1980; Espinoza-Medina *et al.*, 2006; Gallegos-Robles *et al.*, 2008; Morales-Hernández *et al.*, 2009; Jechalke *et al.*, 2019). Such contaminated food contributes to an increasing risk of salmonellosis because it is generally consumed raw. In addition, if *Salmonella* is not controlled early, it can penetrate plant tissues, and subsequent disinfection treatments may be ineffective (Quiroz-Santiago *et al.*, 2009; Freitas *et al.*, 2010; da Cruz *et al.*, 2019).

The prevalence of NTS in RTE food (egg arepa, rice, chicken, corn tortilla, and beef enchilada) and drinking water is low, but could still be risky because they are not often subjected to sanitation processes before ingestion. NTS has an infectious dose as low as 10 cells, depending on the ingested food (Finn *et al.*, 2013). The lack of knowledge and hygiene standards in the processing and sale of street food, common in Latin American countries, can also increase the presence of pathogens, such as *Salmonella* (Valadez *et al.*, 2017).

In our study, the most prevalent serotypes were Enteritidis, Typhimurium and Derby. Enteritidis and Typhimurium were found mainly in chicken and pork respectively, suggesting that these animals constitute their potential reservoirs; these serotypes are associated with a high burden of foodborne *Salmonella* outbreaks in humans (Godínez-Oviedo *et al.*, 2019; Jajere, 2019; Mechesso *et al.*, 2020). Derby has been reported in pork production in Asia, North America, and Europe (Sanchez-Maldonado *et al.*, 2017; Bonardi *et al.*, 2019; Xu *et al.*, 2019); however,

its relationship with human diseases is not fully understood. The high prevalence of Derby in pork products is owing to pigs being the main reservoir of this serotype, and therefore, comprehensive surveillance is needed for its control (Ferrari *et al.*, 2019).

Studies from Chile and Ecuador reported that Infantis is predominant in chicken, suggesting that this serotype is becoming more prevalent than Enteritidis in chicken farming, probably owing to control measures against Enteritidis (Lapierre *et al.*, 2020). In Argentina and Brazil, the predominant serotype was Enteritidis, although the presence of Infantis was also noted in Brazil (Voss-Rech *et al.*, 2015). In Argentina, Infantis was the second most common serotype, isolated also from hospitalized pediatric patients (Merino *et al.*, 2003), which may be a result of its high prevalence in food.

Although sausages undergo preservation processes, NTS has also been reported in this food, suggesting production failures or cross contamination (Escartin *et al.*, 1999). The most frequent serotype found in Brazilian pork sausages was Typhimurium; the source of the pathogen may be animal ingredients because Typhimurium was frequently observed in pork cuts, but it could also be from other ingredients, such as spices (Costa *et al.*, 2020).

Another concern regarding the ubiquity of NTS (food, humans, animals, and the environment) is its AMR, which arises because of the use of antimicrobials in animal husbandry (Silva *et al.*, 2014; McDermott *et al.*, 2018). We found that Typhimurium, Enteritidis and Infantis were the most commonly reported antimicrobial-resistant NTS serotypes, including MDR, 3GCr, and FQNS isolates. These clinically important antimicrobials are used to treat invasive NTS infections (Mechesso *et al.*, 2020).

Our systematic review had some limitations, such as the following. 1) In most studies, the sample size was not statistically calculated, which could have introduced a statistical bias; and 2) some publications were excluded owing to incomplete published data (missing sampling date of the food or unspecified analyzed food).

Conclusions

Through this systematic review of Latin American publications, we showed that, although there is an increasing number of NTS isolated from fruits and vegetables, animal products are the most common carriers of NTS and MDR NTS. Our data correspond with the notion that the current global health problem of AMR is partially related to antimicrobial use in animal husbandry. Therefore, it is critical for Latin America to implement good agricultural practices, reduce the use of antimicrobials in animal husbandry, and implement proper waste management regulations. In addition, an integrated epidemiological surveillance system with the participation of both governmental and non-governmental institutions that handle data regarding food and clinical samples will improve food safety programs.

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Tables (Chapter 1)

TABLE 1. PREVALENCE OF NONTYPHOIDAL *SALMONELLA* IN VARIOUS FOOD PRODUCTS FROM LATIN AMERICA

Country	Analyzed foods	Prevalence (%)	References
Argentina			
	Chicken meat and giblets	3.2-20	Jiménez <i>et al.</i> (2002); Favier <i>et al.</i> (2013); Procura <i>et al.</i> (2019)
	Pork meat	4.5	Colello <i>et al.</i> (2018)
	Pork sausages	6.6	Favier <i>et al.</i> (2013)
	Bovine meat	15.9, 34	Curi de Montbrun <i>et al.</i> (1972); Barril <i>et al.</i> (2019)
	Eggs	3.3	Favier <i>et al.</i> (2013)
	Hen feed	4.6	Soria <i>et al.</i> (2017)
	Crushed bone meal	1.3	Harvey and Price (1978)
Brazil			
	Chicken meat	1.6-68	Santos <i>et al.</i> (2000, 2011); Baú <i>et al.</i> (2001); Luiz <i>et al.</i> (2004); Carvalho and Cortez (2005); Hernandez <i>et al.</i> (2005); Tirolli and Da Costa (2006); Tessari <i>et al.</i> (2008); Asensi <i>et al.</i> (2009); Rall <i>et al.</i> (2009); Medeiros <i>et al.</i> (2011); Cossi <i>et al.</i> (2012); Yamaguchi <i>et al.</i> (2013); Minharro <i>et al.</i> (2015); Dias <i>et al.</i> (2016); Tejada <i>et al.</i> (2016); Ristori <i>et al.</i> (2017); Baptista <i>et al.</i> (2018); da Cunha-Neto <i>et al.</i> (2018); Bersot <i>et al.</i> (2019b); Perin <i>et al.</i> (2019)
	Pork meat	0.8-93.3	de Lima <i>et al.</i> (2004); Borowsky <i>et al.</i> (2007); Tessmann <i>et al.</i> (2008); Seixas <i>et al.</i> (2009); Kich <i>et al.</i> (2011); Silva <i>et al.</i> (2012); Moura <i>et al.</i> (2014); Corbellini <i>et al.</i> (2016); Cabral <i>et al.</i> (2017); Neitzke <i>et al.</i> (2017); da Silva <i>et al.</i> (2018); Bersot <i>et al.</i> (2019a); Paim <i>et al.</i> (2019); Viana <i>et al.</i> (2019)
	Bovine meat	0.03-60	Carvalho and Cortez (2005); Yamaguchi <i>et al.</i> (2013); Cossi <i>et al.</i> (2014); Loiko <i>et al.</i> (2016); Volcão <i>et al.</i> (2016); Bier <i>et al.</i> (2018); Monteiro <i>et al.</i> (2018)

Sausages	4.4-12.8	Benetti <i>et al.</i> (2013); Freire <i>et al.</i> (2019); Werlang <i>et al.</i> (2019)
Chicken sausages	16, 26	Carvalho and Cortez (2005); Cabral <i>et al.</i> (2014)
Pork sausages	5.8-66	Castagna <i>et al.</i> (2005); Spricigo <i>et al.</i> (2008a, 2008b); Fai <i>et al.</i> (2011); Lima <i>et al.</i> (2011); Mürmann <i>et al.</i> (2011); Cabral <i>et al.</i> (2014); Ristori <i>et al.</i> (2017); Cavalin <i>et al.</i> (2018)
Other meat ^a	3.4, 30.8	Hofer <i>et al.</i> (2000); Fernandes <i>et al.</i> (2009)
Eggs	1.5-68	Evêncio-Luz <i>et al.</i> (2012); Kottwitz <i>et al.</i> (2013); de Freitas <i>et al.</i> (2014); Galvão <i>et al.</i> (2018)
Charqui	28	Abrantes <i>et al.</i> (2014)
Dairy products	0.4-40	Yamaguchi <i>et al.</i> (2013); Marinheiro <i>et al.</i> (2015); Reges <i>et al.</i> (2017); de Souza <i>et al.</i> (2017); Queiroz <i>et al.</i> (2018); Carvalho <i>et al.</i> (2019)
Farmed fish, seafood	6.9, 15.2	Vieira <i>et al.</i> (2004); dos Santos <i>et al.</i> (2019)
Vegetables	0.4-25	Takayanagui <i>et al.</i> (2000, 2001); Fröder <i>et al.</i> (2007); de Oliveira <i>et al.</i> (2011); Sant'Ana <i>et al.</i> (2011); Beltrão <i>et al.</i> (2017); Carvalho <i>et al.</i> (2019); da Cruz <i>et al.</i> (2019); Maffei <i>et al.</i> (2019)
Fruit juice and pulp	1.5-33.3	Ruschel <i>et al.</i> (2001); (Ferreira and Junqueira (2007); Rezende <i>et al.</i> (2016)
Peanuts	2.2	Nascimento <i>et al.</i> (2018)
Spices	67	Costa <i>et al.</i> (2020)
Jelly	20	Carvalho <i>et al.</i> (2019)
Animal feed	2.6-66.7	Albuquerque <i>et al.</i> (1999); Silva <i>et al.</i> (2006); Melo <i>et al.</i> (2011); Moura <i>et al.</i> (2014); Bersot <i>et al.</i> (2019a)
Chile		
Chicken meat and giblets	0.4-24.8	Alexandre <i>et al.</i> (2000); Ulloa <i>et al.</i> (2010); Lapierre <i>et al.</i> (2020)
Eggs	0.09	Alexandre <i>et al.</i> (2000)
Colombia		

	Chicken meat and giblets	12.5-83.3	Espinal <i>et al.</i> (2006); Donado-Godoy <i>et al.</i> (2012, 2015); Rodriguez <i>et al.</i> (2015); Ramírez-Hernández <i>et al.</i> (2017); Quintana-Ospina <i>et al.</i> (2018)
	Pork meat	3.3-8.1	Espinal <i>et al.</i> (2006); Rondón-Barragán <i>et al.</i> (2015)
	Bovine meat	40.5	Espinal <i>et al.</i> (2006)
	Sausages	17.6	Espinal <i>et al.</i> (2006)
	Eggs	2.9, 22.2	Mogollón <i>et al.</i> (2016); Quintana-Ospina <i>et al.</i> (2018)
	Dairy products	1.8-62.9	Espinal <i>et al.</i> (2006); Martinez and Gomez (2013); Soto-Varela <i>et al.</i> (2018)
	Ready-to-eat food	0.3-2.7	Espinal <i>et al.</i> (2006); Forero <i>et al.</i> (2017)
	Drinking water	1.4	Espinal <i>et al.</i> (2006)
Costa Rica			
	Chicken giblets	15	Reuben <i>et al.</i> (2004)
	Bovine meat	3.6	Chaves and Brashears (2015)
	Eggs	18.7, 25.3	Arias <i>et al.</i> (1996)
	Tilapia	2	Morales <i>et al.</i> (2004)
	Animal feed, feedstuffs	1.3-26.7	Molina <i>et al.</i> (2015); Leiva <i>et al.</i> (2019)
Cuba			
	Dairy products	19	Martínez <i>et al.</i> (2013)
Ecuador			
	Chicken meat	55.5-88	Vinueza-Burgos <i>et al.</i> (2019); Mejía <i>et al.</i> (2020a)
	Chicken skin	15-80	Villagómez (2015); Sánchez (2016)
	Bovine meat	66.7	Loayza (2011)
	Sausages	25, 30.6	Campoverde (2015)
	Meat ^b	38.1	Mejia <i>et al.</i> (2020b)
	Eggs	0.01, 2	Sánchez (2013); Solano (2016)
	Dairy products	13.7	Plaza-Ibarra and Morales (2001)

	Seafood	7.1	Gecan <i>et al.</i> (1994)
	Animal feed, feedstuffs	0.5-33	Sánchez (2016); Salazar <i>et al.</i> (2019); Vinueza-Burgos <i>et al.</i> (2019)
Honduras			
	Bovine meat	10.1	Maradiaga <i>et al.</i> (2015)
	Vegetables and fruits	2.1	
Mexico			
	Chicken meat and giblets	1.34-47.7	Bello-Pérez <i>et al.</i> (1990); Zaidi <i>et al.</i> (2006); Miranda <i>et al.</i> (2009); Talavera <i>et al.</i> (2011); Villalpando-Guzmán <i>et al.</i> (2017); Regalado-Pineda <i>et al.</i> (2020)
	Pork meat and giblets	1.8-76	Bello-Pérez <i>et al.</i> (1990); Kuri <i>et al.</i> (1996); Zaidi <i>et al.</i> (2006); Miranda <i>et al.</i> (2009); Villalpando-Guzmán <i>et al.</i> (2017)
	Bovine meat and giblets	1.19-71	Bello-Pérez <i>et al.</i> (1990); Heredia <i>et al.</i> (2001); Zaidi <i>et al.</i> (2006); Miranda <i>et al.</i> (2009); Perez-Montaña <i>et al.</i> (2012); Cabrera-Diaz <i>et al.</i> (2013); Narváez-Bravo <i>et al.</i> (2013a); Rubio <i>et al.</i> (2013); Varela-Guerrero <i>et al.</i> (2013); Martínez-Chávez <i>et al.</i> (2015); Villalpando-Guzmán <i>et al.</i> (2017)
	Charqui and sausages	11.1-60	Bello-Pérez <i>et al.</i> (1990); Kuri <i>et al.</i> (1996); Becerril <i>et al.</i> (2019)
	Chorizo	88.3	Escartin <i>et al.</i> (1999)
	Dairy products	4-30.3	Miranda <i>et al.</i> (2009); Torres-Vitela <i>et al.</i> (2012b); Guzman-Hernandez <i>et al.</i> (2016); Chávez-Martínez <i>et al.</i> (2019)
	Seafood	0.9, 11	Bello-Pérez <i>et al.</i> (1990); Quiñones-Ramírez <i>et al.</i> (2000)
	Vegetables	1.8-31	Gallegos-Robles <i>et al.</i> (2008); Orozco <i>et al.</i> (2008a, 2008b); Quiroz-Santiago <i>et al.</i> (2009); Castro-Rosas <i>et al.</i> (2010, 2011); Castañeda-Ramírez <i>et al.</i> (2011); Avila-Vega <i>et al.</i> (2014); Rangel-Vargas <i>et al.</i> (2015); Gutiérrez-Alcántara <i>et al.</i> (2016); Gómez-Aldapa <i>et al.</i> (2017)
	Vegetable salads	6.8-21.8	Miranda <i>et al.</i> (2009); Gómez-Aldapa <i>et al.</i> (2013a, 2017)
	Vegetable and fruit juice	3.9-14.3	Castillo <i>et al.</i> (2006); Castro-Rosas <i>et al.</i> (2010); Torres-Vitela <i>et al.</i> (2012a); Gómez-Aldapa <i>et al.</i> (2014)

	Fruits	12-40	Espinoza-Medina <i>et al.</i> (2006); Gallegos-Robles <i>et al.</i> (2008); Morales-Hernández <i>et al.</i> (2009); Mba-Jonas <i>et al.</i> (2018)
	Fruit salads and flavored waters	15, 16.6	Valadez <i>et al.</i> (2017)
	Eggs	3, 16	Guzmán-Gómez <i>et al.</i> (2013)
	Chocolate	1.8, 4.5	Torres-Vitela <i>et al.</i> (1995)
	Taco dressing	5	Estrada-Garcia <i>et al.</i> (2004)
	Ready-to-eat food	2, 3.27	Bello-Pérez <i>et al.</i> (1990); Gómez-Aldapa <i>et al.</i> (2013b)
Paraguay	Chicken meat	7	Weiler <i>et al.</i> (2017)
Peru	Chicken meat	28.1	Ruiz-Roldán <i>et al.</i> (2018)
	Pork meat	6.3, 6.6	Salvatierra <i>et al.</i> (2015); Ruiz-Roldán <i>et al.</i> (2018)
	Bovine meat	2.3	Ruiz-Roldán <i>et al.</i> (2018)
	Seafood	50	Gecan <i>et al.</i> (1994)
	Vegetables	10	Muñoz <i>et al.</i> (2013)
Uruguay	Bovine meat	0.4	Bosilevac <i>et al.</i> (2007)
	Eggs	0.005	Betancor <i>et al.</i> (2010)
Venezuela	Chicken meat and giblets	23.2, 91	Rengel and Mendoza (1984); Boscán <i>et al.</i> (2005)
	Bovine meat	3.5-45	Narváez <i>et al.</i> (2005); Narváez-Bravo <i>et al.</i> (2013b); Rodríguez-Roque <i>et al.</i> (2018)
	Seafood	2.0, 38.9	Morillo <i>et al.</i> (2007); Gómez-Gamboa <i>et al.</i> (2012)
	Texturized soybean meal	45	Narváez <i>et al.</i> (2005)

^a Horse and sheep meat.

^b Poultry, pork, beef, veal, lamb, and turkey meat.

TABLE 2. DISTRIBUTION AND DIVERSITY OF NONTYPHOIDAL *SALMONELLA* SEROVARS REPORTED FROM FOOD PRODUCTS IN LATIN AMERICA

Country	Analyzed foods	Serotype	Percentage (%) *	References
Argentina				
	Chicken meat	Enteritidis	59.3	Favier <i>et al.</i> (2013)
	Chicken giblets	Schwarzengrund	81.2	Procura <i>et al.</i> (2019)
	Bovine meat	Newport	40.7	Curi de Montbrun <i>et al.</i> , (1972)
		Typhimurium	22.2	Curi de Montbrun <i>et al.</i> (1972)
	Pork meat	Typhimurium	100	Colello <i>et al.</i> (2018)
	Pork sausage	Anatum	22.2	Favier <i>et al.</i> (2013)
	Crushed bone meal	Anatum	22.7	Harvey and Price (1978)
Brazil				
	Chicken meat and derived food	Enteritidis	25-100	Santos <i>et al.</i> (2000); Baú <i>et al.</i> (2001); Hernandez <i>et al.</i> (2005); Cortez <i>et al.</i> (2006); Tessari <i>et al.</i> (2008); Asensi <i>et al.</i> (2009); Medeiros <i>et al.</i> (2011); Palmeira <i>et al.</i> (2016); Ristori <i>et al.</i> (2017)
		Infantis	35.4	da Cunha-Neto <i>et al.</i> (2018)
		Abony	25.8	da Cunha-Neto <i>et al.</i> (2018)
		Schwarzengrund	85.7	Tejada <i>et al.</i> (2016)
		Albany	100	Luiz <i>et al.</i> (2004)
		Typhimurium	42.9	Perin <i>et al.</i> (2019)
		Heidelberg	38.8	Perin <i>et al.</i> ,(2019)
		Kentucky	26.7	Cortez <i>et al.</i> (2006)
	Chicken meat and giblets	Enteritidis	38.48	Minharro <i>et al.</i> (2015)
	Pork meat	Typhimurium	28.6-50.7	Tessmann <i>et al.</i> (2008); Seixas <i>et al.</i> (2009); Kich <i>et al.</i> (2011); Silva <i>et al.</i> (2012); Cabral <i>et al.</i> (2017); Paim <i>et al.</i> (2019)

	Derby	23.1-35.2	Seixas <i>et al.</i> (2009); Silva <i>et al.</i> (2012); Paim <i>et al.</i> (2019)
	Panama	24.1, 28.5	Kich <i>et al.</i> (2011); Silva <i>et al.</i> (2012)
	Infantis	37.5-100	Tessmann <i>et al.</i> (2008)
Bovine meat	Livingstone	100	Loiko <i>et al.</i> (2016)
	Derby	47.1	Cossi <i>et al.</i> (2014)
	Dublin	41.2	Cossi <i>et al.</i> (2014)
Pork sausages	Typhimurium	47.4, 100	Spricigo <i>et al.</i> (2008); Mürmann <i>et al.</i> (2011); Ristori <i>et al.</i> (2017); Werlang <i>et al.</i> (2019)
	Bredeney	33.3	Castagna <i>et al.</i> (2005)
	Saintpaul	20.8	Castagna <i>et al.</i> (2005)
Eggs	Senftenberg	100	Galvão <i>et al.</i> (2018)
	Enteritidis	82.6, 100	Kottwitz <i>et al.</i> (2013)
	Mbandaka	50	Freitas <i>et al.</i> (2014)
	6,7: z10:-	33.3	Freitas <i>et al.</i> (2014)
Seafood (crabs)	Senftenberg	71.4	Vieira <i>et al.</i> (2004)
	Poona	28.6	Vieira <i>et al.</i> (2004)
	Panama	100	Santos <i>et al.</i> (2019)
Vegetables	Javiana	33.3	Takayanagui <i>et al.</i> (2001)
	Typhimurium	75	Sant'Ana <i>et al.</i> (2011)
	O:47:z4,z23:-	25	Sant'Ana <i>et al.</i> (2011)
Orange juice	Cholerasuis	100	Ruschel <i>et al.</i> (2001)
Peanuts	Miami	50	Nascimento <i>et al.</i> (2018)
Pig feed	Senftenberg	100	Silva <i>et al.</i> (2006)
Chile			
Chicken meat	Infantis	96.7	Lapierre <i>et al.</i> (2020)
	Enteritidis	70, 100	Alexandre <i>et al.</i> (2000); Ulloa <i>et al.</i> (2010)

Colombia	Chicken giblets	Enteritidis	38, 100	Alexandre <i>et al.</i> (2000); Ulloa <i>et al.</i> (2010)
	Eggs	Enteritidis	100	Alexandre <i>et al.</i> (2000)
Colombia	Chicken meat	Uganda	33.3	Espinal <i>et al.</i> (2006)
		Anatum	25	Espinal <i>et al.</i> (2006)
	Pork meat	Derby	22.2, 40	Rondón-Barragán <i>et al.</i> (2015)
		Typhimurium	40	Rondón-Barragán <i>et al.</i> (2015)
		Muenster	44.4	Rondón-Barragán <i>et al.</i> (2015)
		Hvittingfoss	22.2	Rondón-Barragán <i>et al.</i> (2015)
		Uganda	33.3	Espinal <i>et al.</i> (2006)
	Bovine meat	Anatum	33.3	Espinal <i>et al.</i> (2006)
		Anatum	38.9	Espinal <i>et al.</i> (2006)
		Uganda	33.3	Espinal <i>et al.</i> (2006)
	Cold meats	Brandenburg	33.3	Espinal <i>et al.</i> (2006)
		Uganda	22.2	Espinal <i>et al.</i> (2006)
	Eggs	Gaminara	22.2	Espinal <i>et al.</i> (2006)
Enteritidis		100	Mogollón <i>et al.</i> (2016)	
Egg arepa	Derby	100	Espinal <i>et al.</i> (2006)	
Drinking water	Rubislaw	100	Espinal <i>et al.</i> (2006)	
Costa Rica	Meat and bone meal	Give	40	Molina <i>et al.</i> (2015)
Costa Rica	Feedstuff	Havana	20.6	Molina <i>et al.</i> (2015)
		Heidelberg	100	Molina <i>et al.</i> (2015)
Cuba	Meat	Agona	21.1	Puig <i>et al.</i> (2011)
	Dairy products	Infantis	100	Puig <i>et al.</i> (2011)

	Processed food	Enteritidis	40	Puig <i>et al.</i> (2011)
		London	40	Puig <i>et al.</i> (2011)
	Egg-based foods	Enteritidis	62.2	Puig <i>et al.</i> (2011)
	Spices	Typhimurium	100	Puig <i>et al.</i> (2011)
Ecuador				
	Chicken meat and skin	Infantis	100	Vinueza-Burgos <i>et al.</i> (2019); Mejia <i>et al.</i> (2020)
	Chicken skin	Infantis	100	Villagómez (2015)
	Eggs	Enteritidis	100	Sánchez (2013); Solano (2016)
	Poultry feed	Infantis	100	Salazar <i>et al.</i> (2019)
Mexico				
	Chicken meat	Gallinarum	33.1	Villalpando-Guzmán <i>et al.</i> (2017)
		Pullorum	22.3	Villalpando-Guzmán <i>et al.</i> (2017)
	Chicken giblets	Typhimurium	100	Talavera <i>et al.</i> (2011)
	Pork meat	Newport	36.6	Villalpando-Guzmán <i>et al.</i> (2017)
		Anatum	31.7	Villalpando-Guzmán <i>et al.</i> (2017)
		Typhimurium	21.8	Villalpando-Guzmán <i>et al.</i> (2017)
	Bovine meat	Typhimurium	28.8-48	Varela-Guerrero <i>et al.</i> (2013); Martínez-Chávez <i>et al.</i> (2015); Villalpando-Guzmán <i>et al.</i> (2017)
		Anatum	40.7	Villalpando-Guzmán <i>et al.</i> (2017)
		Meleagridis	27.9	Zaidi <i>et al.</i> (2006)
		Give	24.4	Perez-Montaña <i>et al.</i> (2012)
	Pork sausage	Derby	26	Escartin <i>et al.</i> (1999)
	Fruits	Agona	100	Mba-Jonas <i>et al.</i> (2018)
		Typhimurium	100	Gallegos-Robles <i>et al.</i> (2008)
	Fruit juice	Agona	46.2	Castillo <i>et al.</i> (2006)
		Typhimurium	30.8	Castillo <i>et al.</i> (2006)
		Anatum	23.1	Castillo <i>et al.</i> (2006)

	Vegetables juice	Typhimurium	75	Gómez-Aldapa <i>et al.</i> (2014)
		Enteritidis	25	Gómez-Aldapa <i>et al.</i> (2014)
	Vegetables	Typhimurium	23.9-80	Gallegos-Robles <i>et al.</i> (2008); Quiroz-Santiago <i>et al.</i> (2009); Rangel-Vargas <i>et al.</i> (2015); Gutiérrez-Alcántara <i>et al.</i> (2016)
		Enteritidis	20-100	Estrada-Garcia <i>et al.</i> (2004); Gallegos-Robles <i>et al.</i> (2008); Gómez-Aldapa <i>et al.</i> (2014); Rangel-Vargas <i>et al.</i> (2015)
	Chocolate	Agona	100	Torres-Vitela <i>et al.</i> (1995)
		Derby	100	
Paraguay	Chili sauce	Enteritidis	100	Estrada-Garcia <i>et al.</i> (2004)
	Chicken meat	Mbandaka	25	Weiler <i>et al.</i> (2017)
Albany		25		
Peru	Pork meat	Derby	100	Salvatierra <i>et al.</i> (2015)
Uruguay	Bovine meat	Newport	100	Bosilevac <i>et al.</i> (2007)
	Eggs	Derby	67	Betancor <i>et al.</i> (2010)
Venezuela	Chicken meat and giblets	Anatum	32	Rengel and Mendoza (1984)
	Chicken giblets	Heidelberg	31.2, 32	Boscán <i>et al.</i> (2005); Boscán-Duque <i>et al.</i> (2007)

* Only values greater than 20% are included.

Supplementary Material (Chapter 1)

Table S1. Databases and search terms (Via Google)

#	Date of search	Search time	Databse	Search terms	Number of citations
#1	July 19, 2020	12:53:00	Pubmed	" <i>Salmonella</i> " AND ((food OR bread OR cooking OR drink OR feed OR foodstuff OR meal OR meat OR snack) AND (Argentina OR Bolivia OR Brazil OR Chile OR Colombia OR "Costa Rica" OR Cuba OR "Dominican Republic" OR Ecuador OR "El Salvador" OR Guatemala OR Haiti OR Honduras OR Mexico OR Nicaragua OR Panama OR Paraguay OR Peru OR "Puerto Rico" OR Uruguay OR Venezuela))	1251
#2	July 19, 2020	13:03:00	SciELO	#1	247
#3	July 19, 2020	13:20:00	Scopus	" <i>Salmonella</i> " AND ((food OR bread OR cooking OR drink OR feed OR foodstuff OR meal OR meat OR snack) AND (Argentina OR Bolivia OR Brazil OR Chile OR Colombia OR "Costa Rica" OR Cuba OR "Dominican Republic" OR Ecuador OR "El Salvador" OR Guatemala OR Haiti))	598
#4	July 19, 2020	13:22:00	Scopus	" <i>Salmonella</i> " AND ((food OR bread OR cooking OR drink OR feed OR foodstuff OR meal OR meat OR snack) AND (Honduras OR Mexico OR Nicaragua OR Panama OR Paraguay OR Peru OR "Puerto Rico" OR Uruguay OR Venezuela))	366
#5	July 19, 2020	13:29:00	Springer	#1	82
TOTAL					2544

Table S2. Food source, serotype, prevalence, and detection method(s) of nontyphoidal *Salmonella* in various food products from Latin America

Country	Analyzed food	Sampling date / period	Isolated nontyphoidal <i>Salmonella</i>	Sample size (<i>n</i>)	Prevalence (%)	Detection method		Reference
						Classical Microbiology protocol	Molecular method(s)	
Argentina	Chicken carcasses	2000-07 to 2001-07	<i>Salmonella</i> spp.	60	20	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Jiménez <i>et al.</i> , 2002)
Argentina	Chicken meat	2005 to 2011	<i>S. Enteritidis</i> <i>S. Montevideo</i>	115	14.8	Bacteriological Analytical Manual (US-FDA)	PCR and PFGE	(Favier <i>et al.</i> , 2013)
	Pork sausages		<i>S. Anatum</i>	90	6.6			
	Chicken giblets		<i>S. Montevideo</i>	62	3.2			
	Liquid eggs		<i>S. Typhimurium</i>	60	3.3			
Argentina	Hen feed	2011-08 to 2012-04	<i>Salmonella</i> spp.	304	4.6	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Soria <i>et al.</i> , 2017)
Argentina	Pig carcasses, pork meat, minced pork	2012 to 2015	<i>S. Typhimurium</i>	764	4.5	Bacteriological Analytical Manual (US-FDA)	PCR- <i>invA</i> gene	(Colello <i>et al.</i> , 2018)
Argentina	Chicken livers	2015-10 to 2016-05	<i>S. Enteritidis</i> <i>S. Typhimurium</i> <i>S. Schwarzengrund</i>	666	4.8	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Procura <i>et al.</i> , 2017)
Argentina	Raw ground beef	2015-12 to 2017-01	<i>S. Enteritidis</i> <i>S. Senftenberg</i> <i>S. Typhimurium</i>	44	15.9	International guidelines ISO 6579	None	(Barril <i>et al.</i> , 2019)
Argentina	Bovine meat	NA	<i>S. Newport</i> <i>S. Typhimurium</i> <i>S. Cholerasuis</i>	100	34	Pre-enrichment, selective enrichment, culture on	None	(Curi de Montbrun <i>et al.</i> , 1972)

			<i>S. Anatum</i>			selective media, reactivity with <i>Salmonella</i> spp. antiserum		
Argentina	Crushed bone meal	NA	<i>S. Anatum</i> <i>S. Senftenberg</i> <i>S. Kentucky</i> <i>S. Meleagridis</i> <i>S. Montevideo</i> <i>S. Derby</i> <i>S. Minnesota</i> <i>S. Oranienburg</i> <i>S. Panama</i> <i>S. Give</i> <i>S. Newport</i> <i>S. Typhimurium</i> <i>S. Bovismorbificans</i> <i>S. Cubana</i> <i>S. Infantis</i> <i>S. Sandiego</i> <i>S. Bredeney</i> <i>S. Eimsbuettel</i> <i>S. Cerro</i> <i>S. Kaminara</i> <i>S. Java</i> <i>S. Jukestown</i> <i>S. Lexington</i> <i>S. Chester</i> <i>S. Corvalis</i> <i>S. Edinburgh</i> <i>S. Glostrup</i> <i>S. Good</i> <i>S. Grumpensis</i> <i>S. Hartfield</i>	2801	1.3	Pre-enrichment, selective enrichment, culture on selective media, reactivity with <i>Salmonella</i> spp. antiserum	None	(Harvey and Price, 1978)

			S. Newlands S. Saintpaul					
Brazil	Horse meat	1980 to 1982	S. Saintpaul S. Agona S. Typhimurium S. Kaapstad S. Chester S. Sandiego S. Schleissheim S. Haifa S. Kimuenza S. Derby S. Tudu S. Reading S. Azteca S. Aynde S. Ball S. Bochum S. Bredeney S. Fyris S. Infantis S. Oranienburg S. Concord S. Colindale S. Eschweiler S. Nigeria S. Papuana S. Bonn S. Inganda S. Montevideo S. Edinburg S. Grampian	19 238	3.4	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Hofer <i>et al.</i> , 2000)

			S. Kotte S. Norwich S. Oslo S. Newport S. Bardo S. Tshiongwe S. Muenchen S. Albany S. Sunnycove S. Virginia S. Amherstiana S. Bovismorbificans S. Kentucky S. Panama S. Eastbourne S. Ndolo S. Portland S. Claibornei S. Jaffna S. Newlands S. Anatum S. Give S. Meleagris S. Harrisonburg S. Nyborg S. Florian S. Langensalza S. Minneapolis S. Newbrunswick S. Ohlstedt S. Vejle S. Fann				
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			S. Rubislaw S. Poona S. Leiden S. Bristol S. Havana S. Roodepoort S. Washington S. Carrau S. Horsham S. Malakal S. Cerro S. Langenhorn S. Minnesota S. Ruiru S. Baguida S. Adelaide					
Brazil	Lettuce, fennel	1995-08 to 1997-07	S. Abaetetuba S. Enteritidis S. Schwarzengrund	NA	3.1	Not indicated	None	(Takayanagui <i>et al.</i> , 2000)
Brazil	Unpasteurized orange juice	1996-03 to 1998-01	S. Cholerasis	52	1.92	Compendium of methods for the microbiological examination of foods, APHA	None	(Ruschel <i>et al.</i> , 2001)
Brazil	Chicken products (chest, back, thighs, wings, heart, liver, gizzard, and eggs)	1997-05 to 1998-04	S. Enteritidis S. Anatum S. enterica subsp. enterica serovar 3,10:e,h:-	124	10.48	Bacteriological Analytical Manual (US-FDA)	None	(Baú <i>et al.</i> , 2001)
Brazil	Lettuce, chicory, arugula	1997-07 to 1998-07	S. Javiana S. Oranienburg S. Anatum	172	9	Pre-enrichment, selective enrichment, culture on	None	(Takayanagui <i>et al.</i> , 2001)

			S. Emek S. Infantis S. Morehead S. Panama S. Typhimurium S. enterica subsp. diarizonae 6:i:z S. Agona			selective media, biochemical characterization		
Brazil	Chicken carcasses	1998-03 to 1998- 05	<i>Salmonella</i> spp.	60	50	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Tirolli and Da Costa, 2006)
Brazil	Chicken carcasses	1998 to 2002	<i>Salmonella</i> spp.	45	13.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Carvalho and Cortez, 2005)
	Mechanically deboned meat			15	25			
	Chicken sausages			25	16			
	Chicken chest			20	30			
	Chicken leg, and thigh			15	13.3			
Brazil	Minced pork	1999 to 2000	<i>Salmonella</i> spp.	30	93.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Borowsky <i>et al.</i> , 2007)
Brazil	Crabs (<i>Ucides cordatus</i>)	2003-02 to 2003- 05	S. Senftenberg S. Poona	90	15.2	Compendium of methods for the microbiological examination of foods, APHA	None	(Vieira <i>et al.</i> , 2004)
Brazil	Eggs (pooled yolks)	2003-08 to 2006- 12	S. Enteritidis	50	6	AOAC Protocol	None	(Kottwitz <i>et al.</i> , 2013)
	Eggs (rinses of eggshells)		S. Enteritidis S. Heidelberg	50	46			

			S. Mbandaka S. Anatum					
Brazil	Lettuce, watercress, spinach, arugula, chicories, broccoli, cabbage, kale, mustard leaves, acelga	2004-03 to 2004- 07	<i>Salmonella</i> spp.	133	3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Fröder <i>et al.</i> , 2007)
Brazil	Chicken carcasses	2004-09 to 2006- 07	S. Enteritidis S. Infantis S. Typhimurium S. Heidelberg S. Mbandaka S. Agona S. Rissen S. Give S. Panama S. Schwarzengrund S. Senftenberg S. Minnesota S. Saintpaul S. Ohio S. Lexington S. Newport S. Gaminara S. Rubislaw <i>Salmonella</i> spp.	2679	2.7	Bacteriological Analytical Manual (US-FDA)	None	(Medeiros <i>et al.</i> , 2011)

Brazil	Fresh pork sausages	2005-03 to 2005-08	<i>S. Typhimurium</i>	125	12.8	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Spricigo <i>et al.</i> , 2008)
Brazil	Fresh pork sausages	2005-09 to 2006-04	<i>S. Derby</i> <i>S. Schwarzengrund</i> <i>S. Typhimurium</i>	200	27	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization, reactivity with <i>Salmonella</i> spp. antiserum	None	(Spricigo, Matsumoto, Espíndola and Ferraz, 2008)
Brazil	Cooked ham	2005-09 to 2005-12	<i>Salmonella</i> spp.	40	30	Compendium of methods for the microbiological examination of foods, APHA	None	(Fai <i>et al.</i> , 2011)
Brazil	Chicken meat	2006-03 to 2006-08	<i>Salmonella</i> spp.	50	8	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Rall <i>et al.</i> , 2009)
Brazil	Pork chuleta	2006-06 to 2006-08	<i>S. Infantis</i>	15	80	Compendium of methods for the microbiological examination of foods, APHA	None	(Tessmann <i>et al.</i> , 2008)
	Pork leg		<i>S. Typhimurium</i> <i>S. Panama</i> <i>S. Typhimurium</i> <i>S. Infantis</i> <i>S. Derby</i>	15				
	Pork rib		<i>S. Infantis</i>	15				
Brazil	Chicken carcasses	2006-07 to 2007-06	<i>S. Enteritidis</i>	116	2.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Tessari <i>et al.</i> , 2008)
Brazil	Sheep meat	2006-09 to 2007-06	<i>Salmonella</i> spp.	26	30.8	ICMSF	None	(Fernandes <i>et al.</i> , 2009)

Brazil	Poultry carcasses	2007-01 to 2013-04	<i>Salmonella</i> spp.	340	19.41	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Bersot, Viana <i>et al.</i> , 2019)
Brazil	Chicken feet	2009-05 to 2009-07	<i>S. Schwarzengrund</i> <i>S. Anatum</i> <i>S. Corvallis</i>	80	68 (before scalding) and 5.6 (after processing)	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Santos <i>et al.</i> , 2011)
Brazil	Charqui (dehydrated meat)	2009-06 to 2019-02	<i>Salmonella</i> spp.	25	28	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Abrantes <i>et al.</i> , 2014)
Brazil	Chicken carcasses	2010-03 to 2010-09	<i>Salmonella</i> spp.	30	3.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Cossi <i>et al.</i> , 2012)
Brazil	Chicken carcasses, heart, and liver	2010-08 to 2011-06	<i>S. Agona</i> <i>S. Anatum</i> <i>S. Enteritidis</i> <i>S. Infantis</i> <i>S. Mbandaka</i> <i>S. Minnesota</i> <i>S. Orion</i> <i>S. Panama</i> <i>S. Schwarzengrund</i>	1500	18.33	MAPA protocol (BRASIL, 2003a)	None	(Minharro <i>et al.</i> , 2015)
Brazil	Cattle carcasses	2010-09 to 2012-02	<i>S. Livingstone</i>	108	0.93	International guidelines ISO 6579	PCR- <i>invA</i> gene	(Loiko <i>et al.</i> , 2016)
Brazil	Pig carcasses	2011-08 to 2012-09	<i>Salmonella</i> spp.	1150	8.7	VIDAS® SLM system (with confirmation)	None	(Corbellini <i>et al.</i> , 2016)

Brazil	Swine carcasses	2011-10 to 2011-11 and 2012-02 to 2012-03	<i>Salmonella</i> spp.	240	29.17	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Silva <i>et al.</i> , 2018)
Brazil	Chicken sausages	2011-11 to 2012-06	<i>Salmonella</i> spp.	29	20	Conventional method and system mini-Vidas®	None	(Cabral <i>et al.</i> , 2014)
	Pork sausages			51	29			
Brazil	Gills of river salmon	2012 to 2015	<i>S. Panama</i>	567	6.87	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	MLST	(Santos <i>et al.</i> , 2019)
Brazil	Chicken carcasses	2014-01 to 2014-09	<i>Salmonella</i> spp.	277	24.5	International guidelines ISO 6579	PFGE	(Dias <i>et al.</i> , 2016)
Brazil	Chicken carcasses	2014-01 to 2015-05	<i>S. Abony</i> <i>S. Agona</i> <i>S. Anatum</i> <i>S. Infantis</i> <i>S. Schwarzengrund</i> <i>Salmonella</i> (O:4,5) <i>Salmonella</i> (O:6,7)	850	3.7	International guidelines ISO 6579	Multiplex-qPCR	(Cunha-Neto <i>et al.</i> , 2018)
Brazil	Peanuts (during processing)	2014-01 to 2015-07	<i>S. Miami</i> <i>S. Muenster</i> <i>S. Javiana</i> <i>S. Oranienburg</i> <i>S. Glostrup</i>	414	2.2	International guidelines ISO 6579	None	(Nascimento <i>et al.</i> , 2018)
Brazil	Custard apple pulp	2014-03 to 2014-08	<i>S. enterica</i> <i>Salmonella</i> spp.	200	1.5	International guidelines ISO 6579	PFGE	(Rezende <i>et al.</i> , 2016)

Brazil	Swine carcasses	2014-04 to 2014-08	<i>Salmonella</i> spp.	258	7.75	AOAC Protocol	No	(Neitzke <i>et al.</i> , 2017)
Brazil	Swine carcasses	2014-05 to 2015-08	<i>S. Typhimurium</i> <i>S. Abony</i> <i>S. Give</i> <i>S. enterica</i> subsp. <i>enterica</i> O:4,5 <i>S. Heidelberg</i>	344	10.5	Bacteriological Analytical Manual (US-FDA)	None	(Cabral <i>et al.</i> , 2017)
Brazil	Fresh cheese	2015	<i>Salmonella</i> spp.	350	25.9	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Carvalho <i>et al.</i> , 2019)
	Handmade jelly				20			
	Cucumber				24.7			
Brazil	Pork sausages	2015-03 to 2015-07	<i>Salmonella</i> spp.	46	28.3	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Cavalin <i>et al.</i> , 2018)
Brazil	Wings	2015-08 to 2016-02	<i>S. Typhimurium</i> <i>S. Heidelberg</i> <i>S. Ndolo</i> <i>S. Minnesota</i> <i>S. enterica</i> subsp. <i>enterica</i> (O:4,5) <i>S. Thompson</i> <i>S. Schwarzengrund</i> <i>S. enterica</i> subsp. <i>enterica</i> (O:3,10:e,h) <i>S. Abony</i>	71	39.4	International guidelines ISO 6579	PCR- <i>invA</i> gene and PFGE	(Perin <i>et al.</i> , 2019)
	Breast			103	32			
	Leg			72	23.6			
	Fried chicken			54	31.5			
Brazil	Fresh cheese	2015-10 to 2016-01	<i>Salmonella</i> spp.	50	40	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Souza <i>et al.</i> , 2017)

Brazil	Sliced collard green	2015-12 to 2017-07	<i>Salmonella</i> spp.	13	23.1	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Cruz <i>et al.</i> , 2019)
	Sliced cabbage			4	25			
Brazil	Chicken carcasses	2016-03 to 2016-09	<i>S. Senftenberg</i> <i>S. Mbandaka</i> <i>S. Schwarzengrund</i> <i>S. Cerro</i> <i>S. Ohio</i> <i>S. Minnesota</i> <i>S. Tennessee</i>	60	26.7	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Baptista <i>et al.</i> , 2018)
Brazil	White cabbage	2016-06 to 2016-08	<i>Salmonella</i> spp.	20	5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Beltrão <i>et al.</i> , 2017)
Brazil	Minced beef	2016-06 to 2017-08	<i>Salmonella</i> spp.	15	27	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Monteiro <i>et al.</i> , 2018)
Brazil	Mozzarella cheese	2016-08 to 2016-10	<i>Salmonella</i> spp.	10	20	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Reges <i>et al.</i> , 2017)
Brazil	Turkey ham	2017-08 to 2017-09	<i>Salmonella</i> spp.	20	10	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Freire <i>et al.</i> , 2019)
Brazil	Black pepper	2018-02 to 2018-07	<i>Salmonella</i> spp.	9	67	Bacteriological Analytical Manual (US-FDA)	None	(Costa <i>et al.</i> , 2020)
Brazil	Chicken meat	5-months period	<i>S. Albany</i>	185	3.24	Pre-enrichment, selective enrichment, culture on	None	(Luiz <i>et al.</i> , 2004)

						selective media, biochemical characterization		
Brazil	Feed ingredients (bone meal, fish's flour, meat meal, blood flour, dry cheese whey base, whey, soybean meal, wafer bran, toasted soy, wheat flour)	NA	S. Heidelberg S. Infantis S. Montevideo S. Mbandaka S. Emek S. Newlands S. Anatum S. Mokola S. Muenster S. Senftenberg S. Cerro	136	19.85	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Albuquerque <i>et al.</i> , 1999)
	Poultry feed			38	2.63			
	Pig feed			5	20			
Brazil	Frozen chicken carcasses	NA	S. Enteritidis S. Agona S. Poona S. Hadar S. Mbandaka S. Anatum S. Havana S. Montevideo S. Ouakam S. Schwarzengrund S. I 4,5,12: -	150	32	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Santos <i>et al.</i> , 2000)

Brazil	Pig carcasses	NA	<i>Salmonella</i> spp.	120	11.7	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Lima <i>et al.</i> , 2004)
Brazil	Pork sausage meat	NA	<i>S. Bredeney</i> <i>S. Typhimurium</i> <i>S. Saintpaul</i> <i>S. Panama</i> <i>S. Enteritidis</i> <i>S. Mbandaka</i> <i>S. Minnesota</i>	38	66	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Castagna <i>et al.</i> , 2005)
Brazil	Chicken legs	NA	<i>S. Enteritidis</i> <i>S. Heidelberg</i>	143	23.8	International guidelines ISO 6579	None	(Hernandez <i>et al.</i> , 2005)
	Chicken breasts		<i>S. Enteritidis</i> <i>S. Thompson</i> <i>S. Munchen</i>	121	11.0			
Brazil	Pig feed	NA	<i>S. Senftenberg</i>	26	7.7	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Silva <i>et al.</i> , 2006)
Brazil	Pequi pulp	NA	<i>Salmonella</i> spp.	10	33.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Ferreira & Junqueira, 2007)
Brazil	Chicken carcasses	NA	<i>S. Enteritidis</i> <i>S. enterica</i> subsp. <i>houtenae</i> <i>S. Mbandaka</i> <i>S. Saintpaul</i> <i>Salmonella</i> spp.	30	47	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Asensi <i>et al.</i> , 2009)
Brazil	Pig carcasses	NA	<i>S. Typhimurium</i> <i>S. Derby</i>	6	4.2	Pre-enrichment, selective enrichment, culture on	None	(Seixas <i>et al.</i> , 2009)

			<i>S. Heidelberg</i>			selective media, biochemical characterization		
Brazil	Pig feed	NA	<i>S. Typhimurium</i> <i>S. Agona</i>	3	66.7	Not indicated	RAPD-PCR (random amplified polymorphism DNA-PCR)	(Melo <i>et al.</i> , 2011)
Brazil	Wild chicory	NA	<i>S. Madelia</i> <i>S. London</i>	13	1.2	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Oliveira <i>et al.</i> , 2011)
Brazil	Swine sausages	NA	<i>Salmonella</i> spp.	91	53	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Lima <i>et al.</i> , , 2011)
Brazil	Minimally processed lettuce	NA	<i>S. Typhimurium</i>	512	0.4	International guidelines ISO 6579	None	(Sant'Ana <i>et al.</i> , 2011)
	Minimally processed arugula		<i>Salmonella enterica</i> subsp. <i>enterica</i> O:47:z4,z23:-					
	Minimally processed organic lettuce and mix of escarole and chicory		<i>S. Typhimurium</i>					
Brazil	Pre-chilled pork carcasses	NA	<i>S. Typhimurium</i> <i>S. Panama</i> <i>S. Derby</i>	98	24	Pre-enrichment, selective enrichment, culture on selective media, further examination	PFGE	(Kich <i>et al.</i> , 2011)
	Post-chilled pork carcasses		<i>S. Typhimurium</i> <i>S. Panama</i>	260	24			

			<i>S. Derby</i> <i>S. Mbandaka</i> <i>S. Infantis</i>					
Brazil	Pork sausages	NA	<i>S. Typhimurium</i>	NA	24.4	Not indicated	None	(Mürmann <i>et al.</i> , 2011)
Brazil	Pig carcasses	NA	<i>S. Typhimurium</i> <i>S. Derby</i> <i>S. Panama</i> <i>S. Brandenburg</i> <i>S. Infantis</i>	109	14.7	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PFGE	(Silva <i>et al.</i> , 2012)
Brazil	Raw eggs from Recife	NA	<i>Salmonella</i> spp.	180	25	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Evêncio-Luz <i>et al.</i> , 2012)
	Raw eggs from Salvador			240	11.25			
	Cheese from Recife			65	7.8			
	Cheese from Salvador			75	12.7			
Brazil	Cold sausages	NA	<i>Salmonella</i> spp.	51	11	Conventional method and system mini-Vidas®	None	(Benetti <i>et al.</i> , 2013)
Brazil	Mechanically separated meat	NA	<i>Salmonella</i> spp.	390	11.02	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Yamaguchi <i>et al.</i> , 2013)
	Chicken carcasses			82	3.66			
	Milk powder			255	1.57			
	Butter			579	0.35			
Brazil	Bovine carcasses	NA	<i>S. Derby</i> <i>S. Dublin</i> <i>S. Infantis</i> <i>S. Give</i> <i>S. salamae</i> subsp. <i>salamae</i>	836	0.7	International guidelines ISO 6579	PCR- <i>ompC</i> gene and PFGE	(Cossi <i>et al.</i> , 2014)

Brazil	Pig feed	NA	<i>S. Typhimurium</i>	3	66.7	International guidelines ISO 6579	RAPD-PCR (random amplified polymorphism DNA-PCR)	(Moura <i>et al.</i> , 2014)
	Pig carcasses		<i>S. Agona</i> <i>S. Infantis</i> <i>S. Minnesota</i> <i>S. Panama</i>	90	25.5			
Brazil	Eggs	NA	<i>S. Mbandaka</i> <i>S. enterica</i> subsp. <i>enterica</i> 6,7: z10:- <i>S. Braenderup</i>	340	1.47	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Freitas <i>et al.</i> , 2014)
Brazil	Sliced mozzarella cheese	NA	<i>Salmonella</i> spp.	20	5	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Marinheiro <i>et al.</i> , 2015)
Brazil	Chicken meat	NA	<i>S. Schwarzengrund</i> <i>S. Mbandaka</i>	200	4	Pre-enrichment, selective enrichment, culture on selective media, further examination	PCR and PFGE	(Tejada <i>et al.</i> , 2016)
Brazil	Ground beef	NA	<i>Salmonella</i> spp.	20	60	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Volção <i>et al.</i> , 2016)
Brazil	Pork sausages	NA	<i>S. Typhimurium</i> <i>S. Derby</i> <i>S. Infantis</i> <i>S. Brandenburg</i> <i>S. Rissen</i> <i>S. I 4,[5],12:i:-</i> <i>S. Ohio</i>	32	62.5	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Ristori <i>et al.</i> , 2017)
	Chicken legs		<i>S. Anatum</i> <i>S. Newport</i> <i>S. Brandenburg</i> <i>S. Enteritidis</i>	32	37.5			

			<i>S. enterica</i> subsp. <i>diarizonae</i> 61:c:z35 <i>S. enterica</i> subsp. <i>diarizonae</i> 61:c:- <i>S. Schwarzengrund</i> <i>S. I 4,[5],12:i:-</i>					
Brazil	Fresh cheese	NA	<i>Salmonella</i> spp.	100	2	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Queiroz <i>et al.</i> , 2018)
Brazil	Eggs (eggshell)	NA	<i>S. Senftenberg</i>	194	1.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Galvão <i>et al.</i> , 2018)
Brazil	Beef carcasses	NA	<i>S. Typhimurium</i>	270	2.6	ISO 2002, with modifications	PCR- <i>invA</i> gene and qPCR	(Bier <i>et al.</i> , 2018)
Brazil	Lettuce	NA	<i>Salmonella</i> spp.	200	1	Pre-enrichment, selective enrichment, culture on selective media, further examination	qPCR	(Maffei <i>et al.</i> , 2019)
Brazil	Salami	NA	<i>S. Typhimurium</i>	90	4.4	ISO 6785/2007	None	(Werlang <i>et al.</i> , 2019)
Brazil	Pig barn feed	2007-01 to 2013-03	<i>S. Typhimurium</i>	216	9.3	USDA Microbiology Laboratory Guidebook (Isolation and Identification of <i>Salmonella</i> from Meat, Poultry and Egg Products)	PFGE	(Bersot, Cavicchioli <i>et al.</i> , 2019)
	Pig carcasses		<i>S. Mbandaka</i> <i>S. Panama</i> <i>S. Derby</i> <i>S. Agona</i> <i>S. Give</i> <i>S. Worthington</i> <i>S. Meleagridis</i> <i>S. Poona</i>	130	0.8			

Brazil	Pork cuts	NA	<i>Salmonella</i> spp.	40	10	International guidelines ISO 6579	PCR- <i>invA</i> and - <i>ompC</i> genes, and PFGE	(Viana <i>et al.</i> , 2019)
Brazil	Pig carcasses (after bleeding)	NA	<i>S. Typhimurium</i> <i>S. Derby</i> <i>S. Brandenburg</i> <i>S. Panama</i>	50	16	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PFGE and WGS-MLST	(Paim <i>et al.</i> , 2019)
	Pig carcasses (before chilling)		<i>S. Derby</i> <i>S. O:4,5</i> <i>S. Typhimurium</i> <i>S. Infantis</i>	50	8			
Chile	Eggs	1998 to 1999	<i>S. Enteritidis</i>	1081	0.09	Bacteriological Analytical Manual (US-FDA)	None	(Alexandre <i>et al.</i> , 2000)
	Chicken meat		<i>S. Enteritidis</i> <i>S. Heidelberg</i>	1154	8.31			
	Chicken giblets		<i>S. Hadar</i> <i>S. Cerro</i> <i>S. Infantis</i> <i>S. Anatum</i> <i>S. Dublin</i>	370	12.97			
Chile	Broiler meat	2016	<i>S. Infantis</i> <i>S. Enteritidis</i>	361	24.8	VIDAS <i>Salmonella</i> (Biomeriux®) International guidelines ISO 6579	PCR target genes amplification	(Lapierre <i>et al.</i> , 2020)
Chile	Chicken carcasses	NA	<i>S. Enteritidis</i>	280	1.8	Not indicated	None	(Ulloa <i>et al.</i> , 2010)
	Chicken giblets			280	0.4			
Colombia	Beef	2002-01 to 2003-03	<i>S. Anatum</i> <i>S. Brandenburg</i> <i>S. Gaminara</i> <i>S. Isangi</i>	1300	40.5	Bacteriological Analytical Manual (US-FDA)	PCR- <i>invA</i> gene	(Espinal <i>et al.</i> , 2006)
	Sausages				17.6			
	Chicken meat				16.2			
	Cheese				12.2			

	Pork meat		<i>S. Muenchen</i>		8.1			
	Egg arepa		<i>S. Newport</i>		2.7			
	Drinking water		<i>S. Rubislaw</i>		1.4			
	Giblets		<i>S. Sandiego</i> <i>S. Typhimurium</i> <i>S. Uganda</i>		1.4			
Colombia	Raw milk	2008-02 to 2008-06	<i>Salmonella</i> spp.	68 (summer) 111 (winter)	4.4 1.8	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Martinez & Gomez, 2013)
Colombia	Poultry meat	2009-03 to 2009-10	<i>S. Heidelberg</i> <i>S. Enteritidis</i> <i>S. Typhimurium</i> <i>S. Muenster</i> <i>S. Lome</i>	200	26	Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) Protocol	None	(Donado-Godoy <i>et al.</i> , 2015)
Colombia	Chicken meat	2010-10 to 2011-04	<i>Salmonella</i> spp.	1003	27	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Donado-Godoy <i>et al.</i> , 2012)
Colombia	Artisanal cheese	2013-01 to 2013-05	<i>Salmonella</i> spp.	27	62.9	Not indicated	qPCR	(Soto-Varela <i>et al.</i> , 2018)
Colombia	Poultry carcasses	2014-02 to 2014-05	<i>S. Hvitvingfoss</i> <i>S. Muenster</i> <i>S. Typhimurium</i> <i>S. Newport</i> <i>S. Heidelberg</i> <i>S. Braenderup</i> <i>S. Kalina</i> <i>S. Bovismorbificans</i> <i>S. Budapest</i> <i>S. Manhattan</i>	270	17.41	International guidelines ISO 6579	None	(Rodriguez <i>et al.</i> , 2015)

			<i>S. Othmarschen</i> <i>S. Schwarzengrund</i> <i>S. Skansen</i>					
Colombia	Eggs (surface)	2014-01 to 2014-08	<i>S. Enteritidis</i>	341	2.93	International guidelines ISO 6579	None	(Mogollón <i>et al.</i> 2016)
Colombia	Chicken carcasses	2015-04 to 2015-11	<i>Salmonella</i> spp.	520	12.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	LAMP (3M MDS)	(Ramírez-Hernández <i>et al.</i> , 2017)
Colombia	Chicken meat	2016-05 to 2016-11	<i>Salmonella</i> spp.	18	83.3	International guidelines ISO 6579	SDS-PAGE and Western blot	(Quintana-Ospina <i>et al.</i> , 2018)
	Eggs (surface)			18	22.2			
Colombia	Pork meat	NA	<i>S. Derby</i> <i>S. Typhimurium</i> <i>S. Javiana</i> <i>S. Muenster</i>	421	3.32	International guidelines ISO 6579	None	(Rondón-Barragán <i>et al.</i> , 2015)
	Pork carcasses		<i>S. Derby</i> <i>S. Muenster</i> <i>S. Hvitvingfoss</i> <i>S. Kattbus</i>		16			
Colombia	Ready to eat rice	NA	<i>Salmonella</i> spp.	303	0.3	Not indicated	qPCR	(Forero <i>et al.</i> , 2017)
	Ready to eat chicken			80	2.5			
Costa Rica	Eggshell	1993-07 to 1994-03	<i>Salmonella</i> spp.	150	25.3	Bacteriological Analytical Manual (US-FDA)	None	(Arias <i>et al.</i> , 1996)
	Egg contents (albumen and yolk)				18.7			
Costa Rica	Meat and bone meal (feedstuffs)	2009 to 2014	<i>S. Anatum</i> <i>S. Brandenburg</i> <i>S. Havana</i>	86	26.74	ISO 17025 accredited modified version of the U.S. Food and Drug Administration	None	(Molina <i>et al.</i> , 2015)

			<i>S. Infantis</i> <i>S. Manchester</i> <i>S. Mbandaka</i> <i>S. Montevideo</i> <i>S. Soerenga</i> <i>S. Muenster</i> <i>S. Yoruba</i> <i>S. Rissen</i> <i>S. Give</i>			Bacteriological Analytical Manual (BAM) method (FDA)		
	Poultry compound feed		<i>S. Amsterdam</i> <i>S. Berta</i> <i>S. Alachua</i> <i>S. Mbandaka</i> <i>S. Anatum</i> <i>S. Senftenberg</i> <i>S. Yoruba</i> <i>S. Rissen</i> <i>S. Schwarzengrund</i> <i>S. Soerenga</i> <i>S. Havana</i>	1420	5.35			
	Pet food		<i>S. Heidelberg</i>	89	3.37			
Costa Rica	Retail beef cuts	2012-08 to 2013-08	<i>Salmonella</i> spp.	279	3.6	Pre-enrichment, selective enrichment, culture on selective media, further examination	PCR target genes amplification	(Chaves and Brashears, 2015)
Costa Rica	Puppy food	2012 to 2018	<i>Salmonella</i> spp.	68	1.47	ISO 17025	None	(Leiva <i>et al.</i> , 2019)
	Adult dog food			158	1.26			
	Cat food			25	4			
Costa Rica	Chicken giblets	NA	<i>Salmonella</i> spp.	100	15	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Reuben <i>et al.</i> , 2004)

Costa Rica	Tilapia	NA	<i>Salmonella</i> spp.	50	2	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Morales <i>et al.</i> , 2004)
Cuba	Artisan fresh cheese	2011	<i>Salmonella</i> spp.	73	19	International guidelines ISO 6579	None	(Martínez <i>et al.</i> , 2013)
Ecuador	Frozen shrimp	1989 to 1990	<i>Salmonella</i> spp.	28	7.1	Not indicated	None	(Gecan <i>et al.</i> , 1994)
Ecuador	Beef	2010-06 to 2010-07	<i>Salmonella</i> spp.	120	66.7	Norma Técnica Ecuatoriana NTE INEN 1529 -15:2009	None	(Loayza, 2011)
Ecuador	Fresh cheese	2011-08	<i>Salmonella</i> spp.	51	13.7	Norma Técnica Ecuatoriana NTE INEN 1529 -15:2009	None	(Plaza-Ibarra and Morales, 2001)
Ecuador	Poultry, pork, beef, veal, lamb, turkey meat	2015 to 2016	<i>S. Infantis</i> <i>S. Typhimurium</i> <i>S. Dublin</i> <i>S. Gallinarum</i> <i>S. Choleraesuis</i> <i>S. Enteritidis</i> <i>S. Javiana</i> <i>S. Heidelberg</i> <i>S. Brandenburg</i> <i>S. Stanley</i> <i>S. Derby</i>	1095	38.1	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	LAMP (3M MDS) PCR- <i>invA</i> and - <i>16S rRNA</i> genes Multiplex PCR	(Mejía <i>et al.</i> , 2020)
Ecuador	Poultry feed	2017-08 to 2017-11	<i>S. Infantis</i>	18	33	International guidelines ISO 6579	PCR target genes amplification	(Salazar <i>et al.</i> , 2019)
Ecuador	Chicken carcasses	2017-11 to 2018-11	<i>S. Infantis</i>	335	55.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	WGS-MLST	(Mejía <i>et al.</i> , 2020)

Ecuador	Eggs	NA	<i>S. Enteritidis</i>	150	0.01	International guidelines ISO 6579	None	(Sánchez, 2013)
Ecuador	Blood sausage	NA	<i>Salmonella</i> spp.	4	25	3M Petrifilm <i>S. Express</i> System	None	(Campoverde, 2015)
	Chorizo		<i>Salmonella</i> spp.	13	30.6			
Ecuador	Chicken skin	NA	<i>S. Infantis</i>	75	80	International guidelines ISO 6579	None	(Villagómez, 2015)
Ecuador	Chicken feed raw materials	NA	<i>Salmonella</i> spp.	177	0.6	International guidelines ISO 6579	PFGE	(Sánchez, 2016)
	Chicken skin before chiller			20	28.3			
	Chicken skin after chiller			20	15			
Ecuador	Eggshells	NA	<i>S. Enteritidis</i>	99	2	Immunochromatography (kit Reveal 2.0 for <i>Salmonella</i>)	None	(Solano, 2016)
Ecuador	Raw feed materials (poultry meat meal, hydrolyzed fish meal, rice, wheat bran, palm kernel cake, soy, corn, rice meal)	NA	<i>S. Infantis</i> <i>S. Mbandaka</i> <i>S. Amsterdam</i>	177	0.5	International guidelines ISO 6579	ERIC-PCR and PFGE	(Vinueza-Burgos <i>et al.</i> , 2019)
	Chicken carcass (after washing)			25	88			
	Chicken carcass (after chilling)			25	56			
Honduras	Beef carcasses		<i>S. Typhimurium</i>	555	10.1			

			<i>S. Derby</i>					
	Fresh vegetables and fruits (cantaloupes, cilantro, cucumbers, leafy greens, peppers, tomatoes)	2011-02 to 2013-01	<i>Salmonella</i> spp.	573	2.1	Pre-enrichment, selective enrichment, culture on selective media, further examination	BAX System PCR Assay	(Maradiaga <i>et al.</i> , 2015)
Mexico	Chorizo and sausage	1986-05 to 1987-04	<i>Salmonella</i> spp.	89	26.45	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Bello-Pérez <i>et al.</i> , 1990)
	Beef			54	16.07			
	Pork			47	13.99			
	Charqui			43	12.80			
	Chicken meat			31	9.22			
	Chicken liver			18	5.36			
	Whole fish			16	4.76			
	Beef enchilada			11	3.27			
	Ground pork			7	2.08			
	Ground beef			7	2.08			
	Pork giblets			6	1.80			
	Beef liver			4	1.19			
	Fish fillet			3	0.89			
Mexico	Raw pork		<i>S. Derby</i> <i>S. Anatum</i>	50	76	Pre-enrichment, selective enrichment, culture on	None	(Kuri <i>et al.</i> , 1996)

	Chorizo	1994-01 to 1994- 03	<p>S. Bredeney S. Agona S. Heidelberg S. Muenster S. Worthington S. Saintpaul S. Muenchen S. Typhimurium S. Brandenburg S. Give S. Infantis S. Senftenberg S. Eko S. Enteritidis S. Havana S. Kentucky S. Lockleaze S. London S. Mbandaka S. Newbrunswick S. Orion S. Panama S. Roterberg S. Tennessee</p>	50	46	selective media, biochemical characterization		
Mexico	Clams	1998-01 to 1998- 11	<p>S. Anatum S. Choleraesuis S. Edimburg S. Enteritidis S. Typhimurium S. Agona S. Gallinarum</p>	260	11	Bacteriological Analytical Manual (US-FDA)	None	(Quiñones- Ramírez <i>et</i> <i>al.</i> , 2000)

Mexico	Tomatoes	1999 to 2003	<i>Salmonella</i> spp.	681	2.8	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Orozco <i>et al.</i> , 2008)
Mexico	Taco dressing	2000	<i>S. Enteritidis</i>	103	5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Estrada-Garcia <i>et al.</i> , 2004)
Mexico	Pork	2001 to 2002	<i>S. Meleagridis</i> <i>S. Havana</i> <i>S. Agona</i> <i>S. Anatum</i> <i>S. Reading</i> <i>S. Worthington</i> <i>S. Typhimurium</i> <i>S. Adelaide</i> <i>S. Infantis</i> <i>S. Derby</i>	339	58.1	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PFGE	(Zaidi <i>et al.</i> , 2006)
	Beef		<i>S. Meleagridis</i> <i>S. Anatum</i> <i>S. Reading</i> <i>S. Agona</i> <i>S. Worthington</i> <i>S. Cerro</i> <i>S. Havana</i> <i>S. Albany</i> <i>S. Derby</i> <i>S. Infantis</i>	126	54			
	Poultry		<i>S. Albany</i> <i>S. Enteritidis</i> <i>S. Agona</i> <i>S. Meleagridis</i>	295	39.7			

			S. Stanleyville S. Braenderup S. Cannstatt S. Reading S. Adelaide S. Havana					
Mexico	In-field cantaloupe melon	2003-09 to 2004-07	<i>Salmonella</i> spp.	35	25.7	Mexican official method (NOM-114-SSA1-1994)	PCR- <i>oriC</i> gene	(Espinoza-Medina <i>et al.</i> , 2006)
	Packed cantaloupe melon			34	20.6			
Mexico	Tomatoes	2003 to 2004	S. Montevideo S. Newport	906	1.8-10.4	Pre-enrichment, selective enrichment, culture on selective media, further examination	PFGE	(Orozco, Iturriaga <i>et al.</i> , 2008)
Mexico	Raw vegetables (celery, watercress, beet, broccoli, zucchini, white round onion, cilantro, cabbage, cauliflower, spinach, large lettuce, Romaine lettuce, potato, "papaloquelite" or Mexican	2004-10 to 2005-03	S. Typhimurium S. Choleraesuis S. Gallinarum S. Anatum S. Agona S. Edinburg S. Enteritidis S. Pullorum	1700 (100 samples of each vegetable)	5.7	Not indicated	None	(Quiroz-Santiago <i>et al.</i> , 2009)

	cilantro, parsley, Chinese parsley, purslane)							
Mexico	Cantaloupe melon	2005-01 to 2005-04	<i>Salmonella</i> spp.	104	12	Pre-enrichment, selective enrichment, culture on selective media, further examination	PCR- <i>invA</i> gene	(Morales-Hernández <i>et al.</i> , 2009)
Mexico	Cantaloupe melon	2006-04 to 2006-06	<i>S. Typhimurium</i>	15	40	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR-RFLP	(Gallegos-Robles <i>et al.</i> , 2008)
	Chile pepper var. Bell		<i>S. Typhimurium</i> <i>S. Enteritidis</i>	13	31			
Mexico	Poultry meat	2007-01 to 2008-08	<i>Salmonella</i> spp.	116	35.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Miranda <i>et al.</i> , 2009)
	Raw cheese			33	30.3			
	Crude vegetable foods			78	21.8			
	Pork			81	17.3			
	Beef			73	15.1			
Mexico	Beef carcasses	2008-12 to 2009-09	<i>S. Give</i> <i>S. Typhimurium</i> <i>S. Infantis</i> <i>S. Anatum</i> <i>S. Bovismorbificans</i> <i>S. Montevideo</i> <i>S. Havana</i> <i>S. Muenster</i> <i>S. Enteritidis</i> <i>S. Livingstone</i> <i>S. Oranienburg</i> <i>S. Panama</i>	505	15.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	Multiplex PCR for <i>invA</i> and <i>fimA</i> genes	(Perez-Montaña <i>et al.</i> , 2012)

			S. Sinstorf								
Mexico	Bell pepper	2009-05 to 2010-05	<i>Salmonella</i> spp.	62	6.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Avila-Vega <i>et al.</i> , 2014)			
Mexico	Beef cattle carcasses at pre-evisceration	2009-07 to 2009-12	S. Anatum	NA	49	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Narváez-Bravo <i>et al.</i> , 2013)			
	Beef cattle carcasses at pre-cooler		S. Montevideo						S. Tennessee	NA	24.8
	Beef cattle carcasses at cooler		S. Kentucky						S. Muenster	NA	6
			S. Give								
			S. Reading								
			S. Mbandaka								
			S. Meleagridis								
			S. Fresno								
Mexico	Ground beef	2009-09 to 2010-07	S. Anatum S. Agona S. Infantis S. Havana S. Typhimurium S. Derby S. Sinstorf S. Panama S. Brandenburg S. Give S. Rissen S. Saintpaul S. Albany S. Braenderup S. Bredeney S. Kentucky S. Lockleaze	238	56.7	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Cabrera-Diaz <i>et al.</i> , 2013)			

			<p><i>S. Worthington</i> <i>S. Adelaide</i> <i>S. Azteca</i> <i>S. Cannstatt</i> <i>S. Montevideo</i> <i>S. Muenchen</i> <i>S. Muenster</i> <i>S. Reading</i></p>					
Mexico	Ground beef	2010-01 to 2010-12	<p><i>S. Anatum</i> <i>S. Newport</i> <i>S. Typhimurium</i> <i>S. Derby</i></p>	864	29.7	Bacteriological Analytical Manual (US-FDA)	None	(Villalpando-Guzmán <i>et al.</i> , 2017)
	Chicken meat		<p><i>S. Anatum</i> <i>S. Newport</i> <i>S. Typhimurium</i> <i>S. Gallinarum</i> <i>S. Derby</i> <i>S. Pullorum</i></p>	864	47.7			
	Pork meat		<p><i>S. Anatum</i> <i>S. Newport</i> <i>S. Typhimurium</i> <i>S. Derby</i></p>	864	22.5			
Mexico	Beef cattle carcasses	2010	<p><i>S. Typhimurium</i> <i>S. Enteritidis</i></p>	109	8.3	Norma oficial mexicana NOM-114-SSA1-1994	PCR resistant genes	(Varela-Guerrero, <i>et al.</i> , 2013)
Mexico	Fresh cheeses	2011-02 to 2011-06	<i>Salmonella</i> spp.	52	4	Bacteriological Analytical Manual (US-FDA)	None	(Guzman-Hernandez <i>et al.</i> , 2016)
Mexico	Fresh Maradol papaya	2011-05 to 2011-08	<i>S. Agona</i>	388	16	Not indicated	PFGE	(Mba-Jonas <i>et al.</i> , 2018)

Mexico	Raw whole nopalitos	2014-07 to 2015-06	<i>Salmonella</i> spp.	100	30	Bacteriological Analytical Manual (US-FDA)	None	(Gómez-Aldapa <i>et al.</i> , 2017)
	Raw nopalitos cut into small squares			100	30			
	Cooked nopalitos salad			100	10			
Mexico	Red chorizo	2015-10 to 2016-04	<i>Salmonella</i> spp.	15 15 6 9 30	20 60 33.3 11.1 30	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Becerril <i>et al.</i> , 2019)
Mexico	Chicken meat	2016-01 to 2018-12	<i>S. enterica</i>	1160	18	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> and - <i>16S rRNA</i> genes	(Regalado-Pineda <i>et al.</i> , 2020)
Mexico	Wrapped chocolate	NA	<i>S. Agona</i> <i>S. Derby</i>	44	4.5	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization and reactivity with <i>Salmonella</i> spp. antiserum	None	(Torres-Vitela <i>et al.</i> , 1995)
	Nonwrapped chocolate			56	1.8			
Mexico	Chorizo (raw pork sausage)	NA	<i>S. Derby</i> <i>S. Anatum</i> <i>S. Infantis</i> <i>S. Typhimurium</i> <i>S. Brandenburg</i> <i>S. Worthington</i> <i>S. Saintpaul</i> <i>S. Schwarzengrund</i> <i>S. Aequatoria</i> <i>S. Senftenberg</i>	200	88.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Escartin <i>et al.</i> , 1999)

			S. Minnesota S. Give S. Binza S. Ohio					
Mexico	Ground meat	NA	<i>Salmonella</i> spp.	88	11.4	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Heredia <i>et al.</i> , 2001)
Mexico	Orange juice from street vendors	NA	S. Agona S. Typhimurium S. Anatum	49	14.3	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Castillo <i>et al.</i> , 2006)
	Orange juice from public markets			51	3.9			
Mexico	Zucchini fruit	NA	<i>Salmonella</i> spp.	100	10	Bacteriological Analytical Manual (US-FDA)	None	(Castro-Rosas <i>et al.</i> , 2010)
Mexico	Jalapeño pepper	NA	<i>Salmonella</i> spp.	100	12	Bacteriological Analytical Manual (US-FDA)	None	(Castro-Rosas <i>et al.</i> , 2011)
	Serrano pepper			100	10			
Mexico	Chicken liver	NA	<i>S. Typhimurium</i>	520	1.34	Pre-enrichment, selective enrichment, culture on selective media, further examination	ERIC-PCR	(Talavera <i>et al.</i> , 2011)
Mexico	Lettuce	NA	<i>Salmonella</i> spp.	75	13	Pre-enrichment, selective enrichment, culture on selective media, further examination	PCR- <i>invA</i> gene	(Castañeda-Ramírez <i>et al.</i> , 2011)
Mexico	Fresh cheese	NA	<i>Salmonella</i> spp.	200	27	Bacteriological Analytical Manual (US-FDA)	None	(Torres-Vitela <i>et al.</i> , 2012)
Mexico	Fresh carrot juice	NA	<i>Salmonella</i> spp.	280	8.6	Bacteriological Analytical Manual (US-FDA)	None	(Torres-Vitela,

								Gómez <i>et al.</i> , 2012)
Mexico	Beef steak	NA	<i>Salmonella</i> spp.	90	8.9	Pre-enrichment, selective enrichment, culture on selective media, further examination	PCR- <i>invA</i> gene	(Rubio <i>et al.</i> , 2013)
Mexico	Ready-to-eat raw vegetable salad	NA	<i>Salmonella</i> spp.	220	6.8	Bacteriological Analytical Manual (US-FDA)	None	(Gómez-Aldapa <i>et al.</i> , 2013)
Mexico	Corn tortillas	NA	<i>Salmonella</i> spp.	200	2	Bacteriological Analytical Manual (US-FDA)	None	(Gómez-Aldapa, Rangel-Vargas, Cruz <i>et al.</i> , 2013)
Mexico	Fresh raw beetroot (<i>Beta vulgaris</i>) juice	10-week study period in the summer	<i>S. Typhimurium</i> <i>S. Enteritidis</i>	100	4	Bacteriological Analytical Manual (US-FDA)	None	(Gómez-Aldapa <i>et al.</i> , 2014)
Mexico	Eggshell	3-months period	<i>Salmonella</i> spp.	2650	16	BCM, bacterial culture method	CM-n-PCR, combined method of enrichment and nested PCR	(Guzmán-Gómez <i>et al.</i> , 2013)
	Egg yolk				3			
Mexico	Alfalfa sprouts	10-week study period in the summer	<i>S. Typhimurium</i> <i>S. Enteritidis</i>	100	4	Bacteriological Analytical Manual (US-FDA)	None	(Rangel-Vargas <i>et al.</i> , 2015)

Mexico	Beef carcasses	NA	<i>S. Typhimurium</i>	142	18	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Martínez-Chávez <i>et al.</i> , 2015)
	Beef chunks		<i>S. Sinfelf</i>	84	39			
	Ground beef		<i>S. Anatum</i>	65	71			
			<i>S. Infantis</i>					
			<i>S. Agona</i>					
			<i>S. Montevideo</i>					
			<i>S. Rissen</i>					
			<i>S. Derby</i>					
			<i>S. Give</i>					
			<i>S. Azteca</i>					
			<i>S. Havana</i>					
			<i>S. Cannstatt</i>					
			<i>S. Bredeney</i>					
			<i>S. Adelaide</i>					
			<i>S. Kaapstad</i>					
			<i>S. Panama</i>					
			<i>S. Lockleaze</i>					
			<i>S. Mbandaka</i>					
			<i>S. Bonn</i>					
			<i>S. Muenchen</i>					
			<i>S. Muenster</i>					
			<i>S. Brandenburg</i>					
			<i>S. Braenderup</i>					
			<i>S. Reading</i>					
			<i>S. Saintpaul</i>					
			<i>S. Sandiego</i>					
Mexico	Raw carrots	NA	<i>S. Typhimurium</i>	100	11	Bacteriological Analytical Manual (US-FDA)	None	(Gutiérrez-Alcántara <i>et al.</i> , 2016)
			<i>S. Montevideo</i>					
			<i>S. Gaminara</i>					
Mexico	Flavored waters	NA	<i>Salmonella</i> spp.	48	16.6	Pre-enrichment, selective enrichment, culture on	None	(Valadez <i>et al.</i> , 20178)
	Fruit salads			20	15			

						selective media, biochemical characterization		
Mexico	Fresh cheese	NA	<i>Salmonella</i> spp.	90	15.38	Not indicated	qPCR	(Chávez-Martínez <i>et al.</i> , 2019)
Paraguay	Processed and unprocessed chicken meat	2011-10 to 2012-04	<i>S. Mbandaka</i> <i>S. Albany</i>	60	7	International guidelines ISO 6579	None	(Weiler <i>et al.</i> , 2017)
Peru	Frozen shrimp	1989 to 1990	<i>Salmonella</i> spp.	2	50	Not indicated	None	(Gecan <i>et al.</i> , 1994)
Peru	Fresh vegetables (lettuce, cabbage, spinach)	2003-10 to 2004-12	<i>Salmonella</i> spp.	180	10	Pre-enrichment, selective enrichment, culture on selective media, further examination	None	(Muñoz <i>et al.</i> , 2013)
Peru	Swine carcasses	Start 2012-03	<i>S. Derby</i>	300	6.3	International guidelines ISO 6579	None	(Salvatierra <i>et al.</i> , 2015)
Peru	Pork (chop)	NA	<i>Salmonella</i> spp.	30	6.6	International guidelines ISO 6579	PCR- <i>invA</i> gene	(Ruiz-Roldán <i>et al.</i> , 2018)
	Beef (loin)			44	2.3			
	Chicken (leg)			64	28.1			
Uruguay	Eggs	2000 to 2002	<i>S. Enteritidis</i> <i>S. Derby</i> <i>S. Panama</i> <i>S. Gallinarum</i>	12400	0.005	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	RAPD amplification	(Betancor <i>et al.</i> , 2010)
Uruguay	Boneless beef trim	2005-01 to 2005-03	<i>S. Newport</i>	1186	0.4	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Bosilevac <i>et al.</i> , 2007)
Venezuela	Beef meat (carcasses)	2006-10 to 2006-12	<i>S. Saintpaul</i> <i>S. Javiana</i>	80	3.5, 4.4, 11.1 (Plants A, B and C)	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Narváez-Bravo, Rodas-

								González <i>et al.</i> , 2013)
Venezuela	Frozen raw shrimp	2008-12 to 2009-12	S. Saintpaul S. Typhimurium S. Tumodi S. Tennessee S. Kentucky S. Mackley S. Javiana S. Rubislaw S. Poona S. Caracas S. Gaminara S. Kikoma S. Michigan	1022	2.0	Bacteriological Analytical Manual (US-FDA)	None	(Gómez-Gamboa <i>et al.</i> , 2012)
Venezuela	Raw ground beef (in raw kibbeh)	2014	<i>Salmonella</i> spp.	7	43	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	PCR- <i>invA</i> gene	(Rodríguez-Roque <i>et al.</i> , 2018)
Venezuela	Chicken carcasses	NA	S. Anatum S. Bloemfontein S. Schwarzengrund S. Halmstad S. Havana S. Ohio S. Victoria S. Aba S. Typhimurium S. Ball S. Glostrup	45	91	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Rengel & Mendoza, 1984)
Venezuela	Chicken giblets	NA	S. Heidelberg S. Amager	332	23.2	Pre-enrichment, selective enrichment, culture on	None	(Boscán <i>et al.</i> , 2005)

			<i>S. Javiana</i> <i>S. Idikan</i>			selective media, biochemical characterization		
Venezuela	Raw ground beef	NA	<i>S. Schwarzengrund</i> <i>S. London</i> <i>S. Anatum</i> <i>S. Sinstorf</i>	NA	45	Bacteriological Analytical Manual (US-FDA)	None	(Narváez <i>et al.</i> , 2005)
	Ground beef reprocessed		<i>S. Braenderup</i>		9			
	Texturized soybean meal		<i>S. Braenderup</i> <i>S. London</i> <i>S. Tennessee</i>		45			
Venezuela	Fresh crab meat	NA	<i>Salmonella</i> spp.	275	38.9	Pre-enrichment, selective enrichment, culture on selective media, biochemical characterization	None	(Morillo <i>et al.</i> , 2007)

NA: Data not available.

Table S3. Antimicrobial resistance patterns of nontyphoidal *Salmonella* isolated from various food products in Latin America

Country	Analyzed food	Sampling date	Isolated NTS serotype	No. of isolates	Resistance pattern	Method	Number of tested drugs	Interpretation guidelines	Reference	
Argentina	Chicken meat	2005-2011	Enteritidis	NA	AMP, FOS, FR, NAL, TET	Kirby–Bauer	14	CLSI, 2009	(Favier <i>et al.</i> , 2013)	
	Chicken giblets		Montevideo	NA	FR, TET					
	Chicken meat		Montevideo	NA	FR					
	Pork sausages		Anatum	NA	TET					
	Liquid egg		Typhimurium	NA	NEO					
Argentina	Pig carcasses	2012 to 2015	Brandenburg	1	AMP, CHL	Kirby–Bauer	16	CLSI, 2014	(Colello <i>et al.</i> , 2018)	
	Pork meat/minced pork meat		Typhimurium	6	AMP, CEF, FOX, GEN, TET, NAL, SXT, CHL					
			Typhimurium	2	AMP, FOX, GEN, TET					
			Retail market pork meat	Typhimurium	1					AMP, CEF, GEN, TET, NAL, CHL, FOS
			Retail market minced pork	Typhimurium	5					AMP, AMK, TET, NAL
				Agona	1					FOX, GEN, AMK, CST
Argentina	Chicken livers	2015-10 to 2016-05	Enteritidis	9	ERY	Kirby–Bauer	30	CLSI, 2013, 2015	(Procura <i>et al.</i> , 2017)	
			Typhimurium	2	ERY					
			Schwazengrund	39	ERY STR					
Brazil	Raw and pasteurized milk	1994-09 to 1995-06	Montevideo	2	KAN, RIF, STM, TET	Kirby–Bauer	15	NA	(Padilha <i>et al.</i> , 2001)	
Brazil	Broiler carcasses	1995-05 to 1996-04	Enteritidis	80 3 16 1 3	CST, ERY, NOV, TET ENR FOS KAN NEO	Kirby–Bauer	16	CLSI, 2001	(Cardoso <i>et al.</i> , 2006)	

				76 69	NIT SUL				
Brazil	Broiler chicken parts (wings, whole legs, boneless breasts, and backs)	1996-09 to 1996-12	Enteritidis	2 6 2 1 2 5	TET NAL, TET NIT, TET NAL, NIT ENR, NAL, TET NAL, NIT, TET	Kirby–Bauer	12	CLSI, 2003	(Ribeiro <i>et al.</i> , 2007)
			Hadar	3	STR, TET				
			Typhimurium	1	NAL, TET				
Brazil	Chicken products and eggs	1997-05 to 1998-04	Enteritidis	NA	PEN	Kirby–Bauer	11	NA	(Baú <i>et al.</i> , 2001)
	Chicken products and eggs		Anatum	NA	PEN				
Brazil	Pork meat	1999 to 2002	Derby	25 15 16 1 2 1	STR+SPT, SUL, TET SUL, SXT, TET TET CHL, AMP, NAL, TET AMP, NAL, TET SUL, SXT, AMP, NAL, TET	Kirby–Bauer	14	CLSI, 2002	(Michael <i>et al.</i> , 2006)
Brazil	Cold chicken	1995 to 2013	Typhimurium	1 1 1 1	STR STR, SUL SUL AMP, NAL, SXT, CHL, TET, GEN, STR, SUL	Kirby–Bauer	12	CLSI, 2015	(Almeida <i>et al.</i> , 2018)

	Chicken			1	SUL				
				2	CTX, FEP, AMP, SUL, CRO				
	Raw pork sausage			1	SUL				
				1	TET, STR, SUL				
	Lettuce			1	STR, SUL				
	Raw kafta			1	TET, STR, SUL				
	Raw tuscan sausage			1	STR				
	Swine			1	AMP, TET, STR, SUL				
				1	NAL, TET, STR, SUL				
				3	TET, STR, SUL				
				1	AMP, NAL, STR, SUL				
				1	NAL, TET, GEN, STR, SUL				
				1	TET, GEN, STR, SUL				
	Poultry			1	TET, SUL				
1		CTX, ATM, FEP, AMP, SXT, TET, STR, SUL, CRO							
2		SUL							
1		STR, SUL							
1		TET, SUL							
1		SUL							
1		SUL							
1		SUL							
Brazil	Mesenteric lymph nodes of pigs, tonsils from pigs and minced pork	1999-09 to 2001-10	Agona	1	CHL, MIN+TET, SUL, TMP	Kirby-Bauer	17	CLSI, 2002	(Michael <i>et al.</i> , 2006)
				1	CHL, MIN+TET, STR, SPT, SUL, TMP				

					AMP, CHL, KAN, MIN+TET, STR, SUL, TMP				
Brazil	Poultry carcass	1995 to 2003	Enteritidis	7	SUL	Kirby– Bauer	11	CLSI, 2005	(Vaz <i>et al.</i> , 2010)
				6	SXT				
				1	NAL				
	Raw poultry meat			2	SUL, SXT				
				2	SXT				
				1	NAL, SUL				
				2	NAL, SXT				
				1	NAL				
	Poultry viscera			1	SUL				
				5	SXT				
				3	NAL, SUL				
				1	NAL, SXT				
				1	NAL, SUL, SXT				
				2	NAL				
				1	STR, GEN, SUL				
				1	STR, SUL				
	Food related to poultry meat or eggs			2	SUL				
Brazil	Meals with eggs or other poultry product	1995 to 1997	Enteritidis	1	NAL	Kirby– Bauer	12	CLSI, 2001	(Oliveira <i>et al.</i> , 2005)
				2	NIT				
				10	SUL				
				8	TET				
				1	TMP				
				1	SUL, NIT				
				1	SUL, TET, NIT				
	Broiler carcasses			4	SUL				
				14	SUL, NIT				
				2	SUL, STR, TET, NIT				

	Poultry derived products (viscera)			2 1 8 3 1 1 1 1 1 1 1	SUL SUL, NAL SUL, NIT SUL, NIT, NAL SUL, STR, GEN SUL, NIT, NAL, CEF SUL, STR, GEN, NIT SUL, STR, TET, GEN, NIT SUL, STR, NOR, GEN, NIT SUL, STR, NOR, GEN, NIT, NAL CHL, SUL, TMP, STR, GEN, CEF, AMP				
Brazil	Frozen chicken	1998 to 2000	Enteritidis	1	SUL	NA	12	CLSI, 1997	(Wilson, 2004)
			Hadar	6	TET				
			Infantis	2	SUL				
Brazil	Mesenteric lymph node of swine and inguinal lymph node of swine	2000 to 2012	Typhimurium	1	AMP, NAL, LVX, SXT, CHL, CIP, TET	Kirby– Bauer	13	CLSI, 2014	(Almeida <i>et al.</i> , 2016)
				3	AMP, NAL, SXT, CHL, TET				
				1	AMP, NAL, SXT, CHL				
				1	AMP, SXT, CHL, TET				
				1	AMP, SXT, CHL				
				2	AMP, CHL, TET				
				1	NAL, SXT, TET				
				1	AMP, CHL				
				2	AMP, TET				
				4	TET				

Brazil	Pig liver	2000 to 2016	Typhimurium	1	CHL, TET, CIP, GEN, NAL, SXT, AMP, STR	NA	NA	NA	(Monte <i>et al.</i> , 2019)
	Pork		Typhimurium	1	CHL, TET, CIP, GEN, NAL, SXT, AMP, STR				
	Swine stomach		Typhimurium	1	CHL, TET, CIP, NAL, AMP, STR				
	Swine muscle		Infantis	1	CHL, TET, CIP, AMP, STR				
				1	CHL, TET, CIP, NAL, AMP, STR				
	Chicken thigh		Schwarzengrund	1	TET, CRO, CIP, GEN, NAL, TIO, SXT, AMP, STR				
	Chicken wing paddle		Schwarzengrund	1	CIP, NAL				
	Chicken carcass		Schwarzengrund	2	CIP, NAL				
				1	TET, CIP, GEN, NAL, SXT, STR				
				10	TET, CRO, CIP, GEN, NAL, TIO, SXT, AMP, STR				
Chicken feet	Minnesota	2	FOX, TET, CRO, AMC, CIP, NAL, TIO, AMP, STR						
		2	CRO, CIP, GEN, NAL, TIO, AMP, STR						
Chicken breast	Brandenburg	1	FOX, TET, CRO, AMC, CIP, NAL, TIO, AMP						

	Chicken wing		Heidelberg	1	FOX, TET, CRO, AMC, CIP, NAL, TIO, AMP, STR				
Brazil	Raw salami after stuffing and salami after curing	2002 to 2003	Panama	3 1 2	AMP, CHL, STX, TET AMP, CHL, STX, TET, ENR AMP, STX, TET	Kirby–Bauer	11	CLSI, 2002	(Ribeiro <i>et al.</i> , 2007)
	Salami after curing		Brandenburg	1	AMK				
Brazil	Food samples of poultry origin	2002 to 2006	Enteritidis	7	NAL	Kirby–Bauer	6	CLSI, 2006	(Kottwitz <i>et al.</i> , 2011)
Brazil	Poultry carcass	2002 to 2012	Senftenberg	1	AMK, SPT, STR, AMP, SUL, CHL, FLF, TET	Kirby–Bauer	17	CLSI, 2008, 2013	(Mattiello <i>et al.</i> , 2015)
Brazil	Poultry	2003 to 2004	Typhimurium	1	ATM, STR, SUL, SXT, TET	Kirby–Bauer	23	CLSI, 2006	(Fernandes <i>et al.</i> , 2009)
Brazil	Poultry	2003 to 2004	Enteritidis	17	ENR, NAL, SXT, TET	Kirby–Bauer	14	CLSI, 2005	(Ribeiro <i>et al.</i> , 2011)
			Ouakam	19	CHL, GEN, KAN, NAL, STR, TET				
			Alachua	18	ENR, NAL, SXT, TET				
	Salami		Brandenburg	1	ENR, KAN, NAL, STR				
			Panama	6	AMP, CHL, KAN, NAL, STR, SXT, TET				
				3	AMP, CHL, KAN, STR, SXT, TET				
Pork ground meat	Panama	5	AMP, CHL, KAN, STR, SXT, TET						
		6	AMP, SXT, STR, TET						
Brazil	Crabs	2003-02 to 2003-05	Senftenberg	NA	NAL, TET	Kirby–Bauer	17	CLSI, 2003	(Vieira <i>et al.</i> , 2004)
			Poona	NA	NAL, TET				
Brazil	Eggshell's rinse and "Pool" yolk (of discarded hatching eggs)	2003-08 to 2006-12	Enteritidis	6	NAL	Kirby–Bauer	6	CLSI, 2006	(Kottwitz <i>et al.</i> , 2013)

Brazil	Frozen chicken carcasses	2004-09 to 2006-07	Enteritidis	NA	AMP, ATM, CEF, FOX, CRO, TIO, FLF, CHL, STR, GEN, NAL, CIP, ENR, TET, SUL, TMP, SXT, NIT	MIC	18	CLSI, 2004	(Medeiros <i>et al.</i> , 2011)
			Infantis	NA	AMP, ATM, TIO, FLF, STR, GEN, NAL, SUL, TMP, SXT				
			Typhimurium	NA	AMP, ATM, TIO, FLF, STR, GEN, NAL, ENR, TET, SUL, TMP, SXT				
			Heidelberg	NA	AMP, ATM, CEF, FOX, CRO, TIO, FLF, STR, NAL, SUL				
Brazil	Fresh pork sausages	2005-03 to 2005-08	Typhimurium	NA	AMP, NEO, SUL, TET	Kirby–Bauer	14	NA	Spricigo <i>et al.</i> , 2008
Brazil	Fresh pork sausages	2005-09 to 2006-04	Derby	NA	SUL, TET, TOB	Kirby–Bauer	15	CLSI, 2001	Spricigo, Matsumoto, Espíndola and Ferraz, 2008
			Schwarzengrund	NA	SUL				
			Typhimurium	NA	TET, TOB				
Brazil	Pig meat cuts	2006-06 to 2006-08	Typhimurium	NA	TMP, ATM, CIP, CRO, FOX, TET, KAN	Kirby–Bauer	14	CLSI, 1984	(Tessmann <i>et al.</i> , 2008)
			Infantis	NA	TMP, ATM, CIP, CRO, FOX, SUL				
			Panama	NA	AMP, CAR, CHL, TET, KAN				

Brazil	Swine mesenteric lymph nodes, Swine head lymph nodes, Swine tonsils	2007	Bredeney	34 1 2 2 3 2 4	SUL, TMP, TET+MIN, CHL, STR, KAN, AMP SUL, TMP, TET+MIN, CHL, STR, KAN, AMP, FLF TMP, TET+MIN, CHL, KAN TET+MIN, CHL SUL, TET+MIN, CHL, STR, KAN, AMP SUL, TMP, TET+MIN, STR, SPT, AMP TET+MIN, STR	Kirby–Bauer	16	CLSI, 2002, 2003	(Michael <i>et al.</i> , 2008)
Brazil	Chicken carcass	2008 to 2009	Schwarzengrund	1	AMP, CAZ, CRO, CTX, TIO, FEP, NAL, CIP, ENR, TET, STR, SXT	MIC	12	CLSI, 2008, 2011	(Silva <i>et al.</i> , 2013)
	Turkey carcass		Agona	1	AMP, CAZ, CRO, CTX, TIO, FEP, NAL, CIP, ENR, TET, STR, SXT				
	Chicken eggs		Agona	1	AMP, CAZ, CRO, CTX, TIO, FEP, NAL, CIP, ENR, TET, STR, SXT				
Brazil	Pig carcasses	2008 to 2011	Typhimurium	1 4 1 1 5 11 1 6 1	NAL, STR, TET AMP, GEN, KAN, TET AMP, NAL, TET, TMP CHL, STR, SUL, TET AMP, GEN, KAN, NAL, TET AMP, NAL, STR, SUL, TET, TMP AMP, CHL, GEN, STR, SUL, TET	Kirby–Bauer	12	CLSI, 2013	(Lopes <i>et al.</i> , 2015)

				1	AMP, CHL, KAN, STR, SUL, TET AMP, CHL, GEN, NAL, STR, SUL, TET, TMP AMP, CHL, CIP, GEN, NAL, STR, SUL, TET, TMP				
			Enteritidis	1	STR, SUL, TET				
			Derby	18	STR, SUL, TET				
			Infantis	1	AMP, CHL, TET, TMP				
			Panama	4	AMP, CHL, KAN, STR, SUL, TET				
				2	AMP, CHL, KAN, NAL, STR, SUL, TET				
			Ohio	1	AMP, CHL, NAL, STR, SUL, TET				
Brazil	Chicken meat	2009 to 2010	Infantis	14 4 2 3 1 3	AMX SUL TET AMX, CAZ AMX, SUL TET, SUL	Kirby– Bauer	10	CLSI, 2013	(Mendonça <i>et al.</i> , 2020)
Brazil	Chicken meat	2009 to 2012	Albany	1 1	CIP, ENR, NAL, TET, SXT FOX, CIP, ENR, NAL, TET, SXT	Kirby– Bauer	15	CLSI	(Penha <i>et al.</i> , 2019)
			Worthington	1	CIP, NAL, TET, CHL				

			Senftenberg	1	FOX, CIP, ENR, NAL, SXT				
			Kentucky	1	FOX, CIP, NAL, TET, SXT				
				1	FOX, CIP, ENR, NAL, TET, SXT				
Brazil	Chicken meat	2010	Minnesota	1	AMC, CRO, TIO, TET, SXT	Kirby–Bauer	9	CLSI, 2013	(Moura <i>et al.</i> , 2017)
Brazil	Chicken meat	2010 to 2015	Heidelberg	95	AMP, CTX, CAZ, CIP, NAL, SXL, TET	MIC	10	EUCAST, 2017	(van den Berg <i>et al.</i> , 2019)
			1	AMP, CTX, CAZ, CIP, NAL, SXL, TET, GEN					
			1	AMP, CTX, CAZ, CIP, NAL, SXL, TET, CST					
			1	AMP, CTX, CAZ, CIP, NAL, SXL, TET, CHL, TMP					
			1	AMP, CTZ, CAZ, SXL, TET					
			1	SMP, CTX, CIP, NAL, SXL, TET, GEN					
			15	CIP, NAL, SXL, TET, GEN					
			13	CIP, NAL, SXL, TET					
Brazil	Poultry (heart)	2010-08 to 2011-06	Agona	2	SXL, SUL, AMC	Kirby–Bauer	12	CLSI, 2011	(Minharro <i>et al.</i> , 2015)
	Poultry (carcass)		Anatum	1	SXL, SUL, AMC				
	Poultry (heart, liver, and carcass)		Enteritidis	10	SXL, AMC				
	Poultry (heart and liver)		Infantis	2	SXL, SUL, AMC				

	Poultry (heart, liver, and carcass)		Mbandaka	5 3 1	SXL, AMC ENR SUL				
	Poultry (liver)		Minnesota	1	SXL, SUL, AMC				
	Poultry (heart, liver, and carcass)		Orion	1	SXL, GEN, AMC				
	Poultry (liver)		Panama	1	SXL, SUL, AMC				
	Poultry (heart)		Schwarzengrund	1	SXL, AMC				
Brazil	Cattle carcasses	2010-09 to 2012-02	Livingstone	1	AMP, CLI, CEF, FOX, ERY, NAL, VAN	NA	6	CLSI, 2012	(Loiko <i>et al.</i> , 2016)
Brazil	Poultry fragments (breast, right and left legs quarter) from carcasses (collected post-bleeding, post-plucking, and post-chilling)	2011-2012	Corvallis	2 4 4	AMP, CRO, CTX NAL, AMP, CRO, CTX, CIP AMP, ATM, CRO, CTX, FEP	Kirby–Bauer	17	CLSI, 2013	(Yamatogi <i>et al.</i> , 2015)
Brazil	Mayonnaise salad and galinhada	2012-11	Alachua	NA	NAL, CIP	Kirby–Bauer	17	CLSI, 2013	(de Almeida <i>et al.</i> , 2015)
Brazil	Poultry meat	2013-2016	Heidelberg	1	CST	Kirby–Bauer, MIC	9	CLSI, 2008	(Moreno <i>et al.</i> , 2019)
			Derby	1	CST				
			Abony	2	CST				
			Muenchen	1	CST, FLF				
			Schwarzengrund	1 1	CST, FLF CST				
Brazil	Chicken sausage	2014 to 2016	Heidelberg	1	AMP, AMC, CAZ, CTX, CRO, CFX	Kirby–Bauer, MIC	8	CLSI, 2015	(Tiba-Casas <i>et al.</i> , 2019)
Brazil		2014 to 2017	Saintpaul	1	CST, CHL	MIC	13		

	Retail poultry, turkey, pork meat and pork carcasses		Typhimurium	1 2 1 1 1 1	CST, AMP, CIP, GEN, NAL, TET CST, AMP, CHL, CIP, GEN, NAL, SXT, TET, TGC CST, AMP, CIP, NAL CST, AMP, CHL, CIP, GEN, NAL, TET, TGC CST, AMP, AZM, GEN, SXT, TET, TGC CST, AMP, CHL, TET, TGC			EUCAST, 2019	(Rau <i>et al.</i> , 2020)
Brazil	Chicken carcasses	2014-01 to 2015-05	Abony	NA	SUL	Kirby–Bauer	17	CLSI, 2014	(da Cunha-Neto <i>et al.</i> , 2018)
			Agona	NA	SUL				
			Anatum	NA	SUL				
			Infantis	NA	SUL				
			Schwarzengrund	NA	SUL				
Brazil	Chicken cuts (wing, breast, leg) and fried chicken	2015-08 to 2016-02	Typhimurium	4 2	AMP, GEN, DOX, TET, NAL, CRO, FEP, ATM AMP, DOX, TET, NAL, CRO, FEP, ATM	Kirby–Bauer	15	CLSI, 2008, 2013	(Perin <i>et al.</i> , 2019)
			Heidelberg	1 3	AMP, AMC, DOX, TET, NAL, TOB, CRO, FEP, ATM AMP, GEN, DOX, TET, NAL, CRO, FEP, ATM				
			Ndolo	1	AMP, GEN, DOX, TET, CRO, FEP, ATM				
				2	AMP, GEN, DOX, TET, NAL, CRO, FEP, ATM				

Chile	Meat	1975 to 1993	Panama	7	STR, SPT	Kirby– Bauer, MIC	16	NA	(Cordano & Virgilio, 1996)
				61	STR, SPT, SUL, TET				
				2	KAN, STR, SPT, SUL, TET				
				2	AMP, GEN, KAN, STR, SPT, SUL, TET				
	Vegetables			3	STR, SPT				
	7	STR, SPT, SUL, TET							
	Shellfish			9	STR, SPT				
	Goat cheese			2	STR, SPT, SUL, TET				
	Fish meal			12	STR, SPT				
				6	STR, SPT, SUL, TET				
Chile	Food intended for human consumption	2007-06 to 2009-06	Derby	1	AMX, AMP, TIO, ENR, FLF, OXY, SXT	Kirby– Bauer	7	CLSI, 2008	(Junod <i>et al.</i> , 2013)
				1	TIO, OXY, SXT				
Colombia	Poultry, meat, and mixed sources	1997 to 2002	Typhimurium	2	SXT, AMP	Kirby– Bauer	8	CLSI, 2002	(Wiesner <i>et al.</i> , 2006)
				2	SXT, AMP, TET				
				2	SXT, AMP, TET, NAL				
				1	SXT, AMP, TET, CHL				
				1	SXT, TET, NAL				
				2	TET				
				1	TET, GEN				
				1	TET, CTX, AMP				
Colombia	Bovine meat	2002 to 2009	Carrau	1	NAL	Kirby– Bauer, MIC	15	CLSI, 2007	(Karczmarczyk <i>et al.</i> , 2010)
	Cheese			2	NAL				
	Ground meat			1	NAL, TET				
	Cheese		Anatum	1	AMC, AMP, CEF, TET				
				1	STR, TET				
				1	NAL				
Ground meat		1	TET						
Sausages		1	GEN, STR, TET						

Ham		1	NAL				
Cheese		1	STR				
Retail chicken	Infantis	1	KAN, NEO, NAL, STR, TET				
Sausages		1	AMP				
Potato and meat		1	AMP				
Retail chicken		1	STR				
Sausages		1 1	KAN, NEO, NAL, STR, TET TET				
Ground meat	Uganda	1 1 1 1	KAN, NEO, NAL, STR, TET KAN, NEO, TET NAL, TET AMP				
Retail chicken		1	TET				
Ham		1	AMP				
Ground meat		1	AMP				
Sausages	Braenderup	1 1	TET AMP				
Bovine meat		1	CEF				
Bovine meat		1	AMP				
Potato	Saintpaul	1	AMP				
Cheese	Muenchen	1 1 1	NAL TET AMP NAL, TET				
Ham		1	TET				
Meat (bovine)		1	AMP				
Ground meat	Senftenberg	1	AMP				
Sausages	Javiana	1	AMP				
Sausages	Fresno	1	AMP				

	Ground meat		Gaminara	1	AMP				
Colombia	Poultry meat	2009-03 to 2009-10	Heidelberg	2	AMC, AMP, CFZ, FOX, CRO, CTX, TIO, NAL, CIP, ENR, TET, TIL	MIC	21	CLSI, 2012	(Donado-Godoy <i>et al.</i> , 2015)
				3	AMC, AMP, CFZ, FOX, CRO, CTX, TIO, NAL, TET, TIL				
				1	NAL, CIP, ENR, STR, TET, TIL				
				1	NAL, CIP, ENR, TET, TIL				
1	NAL, CIP, TET, TIL								
			Enteritidis	1	TIL, NIT				
				1	TIL, CFZ				
				1	TIL, GEN				
				6	TIL				
			Typhimurium	1	STR, TET, SXT, TIL				
				1	STR, TET, TIL				
				1	STR, TIL				
Colombia	Atoll rice	2010	Typhimurium	11	TET, STR	Kirby–Bauer, MIC	13	CLSI, 2010	(Díaz <i>et al.</i> , 2014)
Colombia	Chicken sandwich (bread, cooked shredded chicken, green tomato, pineapple, and homemade garlic sauce)	2011-03	Enteritidis	1	NAL	Kirby–Bauer, MIC	12	CLSI, 2012	(Díaz <i>et al.</i> , 2013)

Colombia	Raw chicken meat	2011-08 to 2012-05	Heidelberg	2 1 40 23 52 7 47 19 42 21 46 46 54 59 23 40 67 68 35	GEN TOB STR AMC AMP TZP CFZ FEP CTX FOX TIO CRO CIP ENR LVX NIT NAL TET SXT	Kirby– Bauer, MIC	22	CLSI, 2013	(Donado- Godoy <i>et al.</i> , 2014)
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			Enteritidis	8 5 9 1 7 1 7 8 14 7 7 9 5 49 10 4 4	STR AMC AMP TZP CFZ FEP CTX FOX TIO CRO CIP ENR LVX NIT NAL TET SXT				
			Typhimurium	16 1 8 2 6 4 4 6 4 8 9 4 9 12	STR AMC AMP TZP CFZ FEP CTX TIO CRO CIP ENR LVX NIT NAL				

				12 8	TET SXT				
Colombia	Hen feed and eggshells	2013-01 to 2013-06	Shannon	8 2	AMK, CEF, FOX, CXM, GEN SXT	Kirby– Bauer	13	CLSI, 2006	(Rodríguez <i>et al.</i> , 2015)
			Enteritidis	6 5	AMK, CEF, FOX, CXM, GEN NIT				
Colombia	Chicken meat	2014-02 to 2014-05	Newport	2	AMK, CFZ, FOX, GEN, TOB	Kirby– Bauer	19	CLSI, 2015	(Cortes <i>et al.</i> , 2017)
			Skansen	1	AMK, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR				
			Kalina	2	AMK, CFZ, FOX, GEN, TOB				
			Schwarzengrund	1	AMK, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR				

			Manhattan	1	AMK, CFZ, FOX, GEN, TOB				
			Braenderup	2	AMK, CFZ, FOX, GEN, TOB				
			Bovismorbificans	1	AMK, CFZ, FOX, GEN, TOB				
			Typhimurium	1 1	AMK, CFZ, FOX, GEN, TET, TOB, CHL, FLF AMK, CFZ, FOX, GEN, TET, TOB				
			Othmarschen	1	AMK, CFZ, FOX, GEN, TOB				
			Hvittingfoss	1 8	AMK, CFZ, FOX, GEN, TOB, FLF AMK, CFZ, FOX, GEN, TOB				
			Heidelberg	2	AMK, AMP, CFZ, FOX, CIP, GEN, NIT, TET, TOB, SXT, ENR				
			Muenster	5	AMK, AMP, CFZ, FOX, GEN, TET, TOB				
			Budapest	1	AMK, AMP, SAM, CFZ, FOX, CRO, CIP, GEN, NIT, TET, TOB, ENR				
Costa Rica	Poultry compound feed	2009 to 2014	Anatum	NA	TET	Kirby-Bauer, MIC	1	CLSI, 2015	(Molina <i>et al.</i> , 2015)
	Poultry compound feed		Havana	NA	TET				
	Meat and bone meal		Give	NA	TET				

Cuba	Fresh meat	2008-01 to 2009-12	Agona	3 2 1	TET NAL CRO	Kirby- Bauer	9	CLSI, 2008	(Puig <i>et al.</i> , 2011)	
	Fresh meat		Bissau	1	NAL					
	Fresh meat		Derby	2 1	TET AMP					
	Meat products			1	AMP, TET, NAL					
	Fresh meat		Enteritidis	18 1	TET NAL, CIP					
	Egg-based food			4 1	AMP AMP, TET, NAL					
	Meat products			2	NAL					
	Fresh meat		Hato	1	TET					
	Fresh meat		Infantis	3 1	TET AMP, TET, CRO					
	Dairy products			3	AMP					
	Egg-based foods		Kisii	1	TET					
	Fresh meat			1	NAL, TET					
	Fresh meat		London	2 1 1 1	AMP NAL AMP, TET NAL, CRO					
	Fresh meat			Muenchen	2					TET
	Fresh meat			Nchanga	2					TET
	Fresh meat			Shangani	1					TET
	Fresh meat		Typhimurium	4 1 1	TET CRO AMP, TET					
	Egg-based foods			2	AMP					
	Processed meat			Kande	1					TET
	Processed meat		Montevideo	1	TET					

	Meat products		Asylanta	2	TET				
	Meat products		Sinstorf	2	TET				
	Egg-based foods		Irumu	1	TET				
Ecuador	Poultry, pork, beef, veal, lamb, turkey	2015 to 2016	Infantis	86 12 27 11 2 3 12 9 10 8	STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, AMP, KAN STR, CTX, CRO, CIP, NAL, TET, AMP STR, CTX, CRO, CIP, NAL, TET, CHL, AMP, KAN STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP STR, CTX, CRO, CIP, NAL, TET, AMP, KAN STR, SXT, CTX, AMC, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, AMC, CRO, CIP, NAL, TET, CHL, GEN, AMP, AZM, KAN STR, SXT, CTX, CRO, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP, AZM, KAN	Kirby– Bauer	13	CLSI, 2020	(Mejía <i>et al.</i> , 2020)

	Poultry, pork, beef, veal		Dublin	1 1 1 1	AMP, CTX, CRO, CIP, GEN, KAN, NAL, STR, SXT, TET STR, SXT, CTX, AMC, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, AMC, CRO, CIP, NAL, TET, CHL, GEN, AMP, AZM, KAN STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP, AZM, KAN				
	Pork, veal		Typhimurium	1 1 1 1	STR, NAL, TET, CHL STR CIP STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN				
	Beef		Javiana	1	TET				
	Lamb		Heidelberg	2 1 1	STR, SXT, CTX, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, AMC, CRO, CIP, NAL, TET, CHL, GEN, AMP, KAN STR, SXT, CTX, CRO, NAL, TET, CHL, GEN, AMP, KAN				
Ecuador	Poultry feed	2017-08 to 2017-11	Infantis	1 1	TET, NIT, FR, FOS, ENR, CIP, CRO, FEP, AMP, STR	Kirby–Bauer	20	CLSI, 2019	(Sánchez-Salazar <i>et al.</i> , 2019)

				1	TET, SXT, NIT, FR, FOS, CHL, CIP, CRO, FEP, AMP, GEN, STR				
				1	TET, SXT, NIT, FR, FOS, CHL, ENR, CIP, CRO, FEP, AMP, GEN, STR				
				1	TET, SXT, NIT, FR, FOS, CHL, ENR, CIP, CRO,				
				1	CAZ, FEP, AMP, GEN,				
				1	STR TET, NIT, FR, FOSF, CHL, ENR, CIP, CRO, FEP, AMP, GEN, STR				
					TET, NIT, FR, CHL, CIP, GEN, STR				
					TET, CIP				
					TET, SXT, NIT, FR, FOSF, CHL, CIP, CRO, FEP, AMP, GEN, STR				
					TET, SXT, NIT, FR, FOSF, CHL, ENR, CIP, CRO, FEP, AMP, GEN, STR				
					TET, SXT, NIT, FR, FOS, CHL, ENR, CIP, CRO, CAZ, FEP, AMP, GEN, STR				
					TET, NIT, FR, FOS, CHL, ENR, CIP, CRO, FEP, AMP, GEN, STR				
					TET, NIT, FR, CHL, CIP,				

					GEN, STR TET, CIP				
Mexico	Retail chicken, retail pork, retail beef, chicken intestine, swine intestine, bovine intestine	2000 to 2005	Typhimurium	NA	PIP, TIC, FOX, CAZ, CTX, TIO, ATM, SXT, NAL, GEN, KAN	Kirby– Bauer, MIC	11	CLSI, 2000	(Zaidi <i>et al.</i> , 2006)
Mexico	Chicken meat	2002-03 to 2005-08	Typhimurium	22 20 4 19 17 6 12	AMP, STR SF, TET GEN, KAN CHL CRO NAL SXT	Kirby– Bauer, MIC	11	CLSI, 2000	(Zaidi <i>et al.</i> , 2008)
	Pork meat			78 113 2 51 60 52 92 123 118 81 120	AMP CHL CIP CRO GEN KAN NAL STR SF SXT TET				

	Beef			25 38 4 24 19 42 50 48 30 49	AMP CHL CRO GEN KAN NAL STR SF SXT TET				
Mexico	Pork meat and intestine; bovine intestine	2006-03 to 2007-05	Typhimurium	1	AMP, FOX, CAZ, CRO, CHL, NAL, STR, SXT, SF, TET	Kirby–Bauer, MIC	12	CLSI, 2009, 2010	(Zaidi <i>et al.</i> , 2012)
	Beef, chicken meat and intestine, pork meat; fresh fruit beverage		Agona	1 1 1	CHL, STR, SXT, TET, SF SF STR				
Mexico	Beef carcass	2008-12 to 2009-09	Typhimurium	7 5 1	GEN, TET, SXT, CHL, STR, KAN GEN, TET, SXT, CHL, NAL, STR, CIP GEN, TET, SXT, CHL, KAN, STR	Kirby–Bauer	11	CLSI, 2008	(Perez-Montaño <i>et al.</i> , 2012)
			Infantis	1	GEN, TET, SXT, CHL, STR, KAN				
			Havana	1	AMP, TET, SXT, STR, CIP				
Mexico	Ground beef	2009-09 to 2010-07	Anatum	2 1 1	AMP, TET, SXT, CEF, STR, GEN, CHL AMP, TET, NAL, SXT, STR TET, SXT, STR, CHL	Kirby–Bauer	11	CLSI, 2008	(Cabrera-Diaz <i>et al.</i> , 2013)

				1 1	TET, NAL, STR, CHL TET, NAL, STR				
			Agona	1	AMP, TET, SXT, STR				
			Havana	1	TET, SXT, STR				
			Typhimurium	1 3 1	AMP, TET, CEF, STR, GEN, CHL TET, NAL, SXT, STR, CHL AMP, TET, STR				
			Derby	1 1 1	AMP, TET, NAL, SXT, STR TET, SXT, STR, CHL TET, NAL, STR				
			Sinstorf	1	AMP, TET, SXT, STR, CHL				
			Panama	1	AMP, TET, SXT, STR, GEN, CHL				
			Brandenburg	1	AMP, TET, SXT, STR				
			Give	1	AMP, TET, NAL, STR, CHL				
			Rissen	1	TET, NAL, SXT, STR, CHL				
			Saintpaul	1 1	AMP, TET, SXT, STR TET, NAL, STR				
			Reading	1	TET, NAL, STR				
Mexico	Chicken meat	2010-01 to 2010-12	Anatum	27 7 2 1	CAR, AMP, CHL, CTX, PEF GEN SXT	Kirby- Bauer	12	CLSI, 2010	(Villalpando- Guzmán <i>et al.</i> , 2017)

				AMK, CEF, CRO, NET, NIT				
			Newport	16 4 2 1	CAR, AMP, CHL, CTX, PEF GEN CRO, CEF, SXT, NET AMK, NIT			
			Typhimurium	15 2 1	CAR, AMP, CHL, CTX, PEF AMK, CRO, GEN CEF, SXT, NET, NIT			
			Gallinarum	43 30 19 13 10 2 1	AMP CHL CTX CAR, GEN PEF NIT AMK, CRO, CEF, SXT, NET			
			Derby	4 2 1	CAR, AMP, CHL, CTX, PEF CRO, GEN AMK, CEF, SXT, NET, NIT			

Beef meat	Pullorum	27 22 21 15 9 7 2 1	AMP CHL CTX PEF GEN CAR CRO, CEF, NIT AMK, SXT, NET				
	Anatum	48 39 37 27 23 3 2 1	PEF AMP CHL CAR CTX AMK, NIT CRO, GEN, SXT, NET CEF				
	Newport	22 10 4 3 2 1	CAR, AMP, CHL, CTX, PEF CRO CEF GEN AMK, NET, NIT SXT				
	Typhimurium	34 32 5 4 3 2 1	CAR, AMP, CHL, CTX PEF CRO NIT AMK CEF, SXT, NET GEN				

			Derby	14 13 3 2 1	CAR, AMP, CHL, CTX PEF CRO, GEN SXT, NIT AMK, CEF, NET				
	Pork meat		Anatum	32 4 3 2 1	CAR, AMP, CHL, CTX, PEF CRO AMK CEF, GEN SXT, NIT				
			Newport	37 3 2 1	CAR, AMP, CHL, CTX, PEF NIT CRO, GEN, SXT, NET AMK, CEF				
			Typhimurium	22 3 2 1	CAR, AMP, CHL, CTX, PEF CRO, SXT, NIT AMK, CEF, NET GEN				
			Derby	10 3 2 1	CAR, AMP, CHL, CTX, PEF NIT CEF, GEN, SXT AMK, CRO, NET				
			Cannstatt	1	AMP, CAR, TET				
Mexico	Ground beef	2013-04 to 2013-11	Lomita	3 1 1 1	AMP, CAR, SXT, TET AMP, CAR, CEF, SXT, TET AMP, CAR, SXT, PEF, TET	Kirby- Bauer	14	CLSI, 2012	(Nayarit- Ballesteros <i>et al.</i> , 2016)

					AMP, CEF, SXT, GEN, TET				
			Senftenberg	1 1	AMP, CAR, SXT, CHL, TET AMP, CAR, CEF, SXT, CHL, GEN, TET				
			Derby	1 1 1	AMP, CAR, SXT, TET AMP AMP, CAR				
			Javiana	1	AMP, CAR, CEF, CHL, TET				
Uruguay	Eggs	2000 to 2002	Enteritidis	2 4	NAL AMP, CEF, FOX, AMC, SAM, CXM	Kirby– Bauer	13	CLSI, 2001	(Betancor <i>et al.</i> , 2004)
Uruguay	Eggs	2000 to 2002	Panama	2	AMP, CEF, CXM, CAZ	Kirby– Bauer	13	CLSI, 2006	(Betancor <i>et al.</i> , 2010)
			Gallinarum	1 4	AMP NAL				
			Amager	2 4	AMP NAL				
			Idikan	2 2	NAL TET				
			Javiana	4	NAL				
Venezuela	Raw chicken meat	2016	Heidelberg	1 1 1 1 1	AMP, FOX, TET, SXT AMP, FOX, CAZ, ATM, TET, SXT, GEN, CIP AMP, FOX, CAZ, ATM, TET, SXT, GEN, TOB, CIP AMP, FOX, SXT	MIC	14	CLSI, 2018	(Gonzalez and Araque, 2019)

				AMP, FOX, AZM, TET, SXT				
			Enteritidis	1 1	AMP, TET, SXT AMP, SXT			
			Typhimurium	1	AMP, TET			
			Meleagridis	1	TET, SXT, CIP			

NA: Data not available. **MIC:** Minimum inhibitory concentration. **Antimicrobials:** amikacin (AMK), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ampicillin (AMP), ampicillin-sulbactam (SAM), azithromycin (AZM), aztreonam (ATM), carbenicillin (CAR), cefaclor (CEC), cefadroxil (CFR), cefazolin (CFZ), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), ceftiofur (TIO), ceftriaxone (CRO), cefuroxime (CXM), cephalothin (CEF), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), colistin (CST), doxycycline (DOX), enrofloxacin (ENR), erythromycin (ERY), florfenicol (FLF), fosfomycin (FOS), furazolidone (FR), gentamicin (GEN), kanamycin (KAN), levofloxacin (LVX), lincomycin (LIN), minocycline (MIN), nalidixic acid (NAL), neomycin (NEO), netilmicin (NET), nitrofurantoin (NIT), norfloxacin (NOR), novobiocin (NOV), oxytetracycline (OXY), pefloxacin (PEF), penicillin (PEN), piperacillin (PIP), piperacillin-tazobactam (TZP), rifampicin (RIF), spectinomycin (SPT), streptomycin (STR), sulfafurazole (SOX), sulfamethoxazole (SXL), sulfamethoxazole-trimethoprim (SXT), sulfisoxazole (SF), sulphazotrim (STM), sulfonamide (SUL), tetracycline (TET), ticarcillin (TIC), tigecycline (TGC), tilmicosin (TIL), trimethoprim (TMP), Tobramycin (TOB), vancomycin (VAN).

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CHAPTER 2

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***Salmonella* grows massively and aerobically in chicken faecal matter**

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Summary

The use of wastewater for irrigation and animal manure as fertilizer can cause transmission of intestinal pathogens, conditions frequently observed in Low- and Middle-Income Countries (LMICs). Here we tested the ability of *Salmonella* to grow in the faecal matter. We inoculated freshly isolated *Salmonella* strains (from chickens) in chicken faecal matter and incubated for 1 to 12 days, under aerobic and anaerobic conditions. We found that both *Salmonella* and *Escherichia coli* multiplied massively in faecal matter outside a host and significantly higher in aerobic conditions. Our results have critical implications in waste management, as we demonstrate that aerobic treatments may not be the best to reduce the number of *Salmonella* in the environment.

Introduction

Environmental transmission of intestinal pathogens is extremely important especially in Low- and Middle-Income Countries (LMICs) due to deficient sanitary infrastructure, unplanned

urban growth, lack of wastewater treatment, etc. One of the main concerns in LMICs is the large proportion of untreated wastewater used for irrigation (Khalid *et al.*, 2018) and the increasing use of animal manure as fertilizer without suitable treatment (Mandrell, 2009). Reports of grave enteric infections caused by environmental contamination of edible vegetables are also commonplace nowadays in industrialized countries (Callejón *et al.*, 2015). Some of these outbreaks have been associated with high mortality, morbidity, and large economic losses. The incidence of these infections is exacerbated by the increasing appeal to consume natural, non-processed fresh products (Mandrell, 2009).

Salmonella-contaminated water is responsible for a large number of outbreaks by the ingestion of water or produce (Mandrell, 2009); the sources for this contamination are human and non-human faecal matter (Medrano-Félix *et al.*, 2017). The use of animal waste as fertilizer constitutes a serious risk that can be controlled by appropriate composting technology (Tiquia *et al.*, 1998; Szogi *et al.*, 2015;). Human waste contamination, however, is much more difficult to monitor or control in LMICs where wastewater treatment or toilets are not available (Khalid *et al.*, 2018). The fate of *Salmonella* in these conditions is not understood completely, although some researchers indicate that *Salmonella* enters into a viable-non culturable state outside the host (Winfield and Groisman, 2003). The reduction of the risk of this type of transmission requires an understanding of every aspect of *Salmonella* physiology in the environment outside the host (Mandrell, 2009). It is worth mentioning that *Salmonella*'s ability to grow in the faecal matter has been ignored.

It is known that *Salmonella* and other *Enterobacteriaceae* survive in faecal matter for some time and it has been shown that *Escherichia coli* (another member of the *Enterobacteriaceae*) also grows massively in faecal matter (Russell and Jarvis, 2001; Vasco *et al.*, 2015; Sharma *et al.*, 2019). Here we tested *Salmonella*'s ability to grow in faecal matter in aerobic and anaerobic conditions and discuss the potential implications for faecal waste management.

Results and discussion

Two trials were performed with *Salmonella* Infantis inoculated in chicken faecal matter. In the first trial we determined the growth of *Salmonella* by plate counting and by molecular detection after 0, 24, 48 and 72 h of incubation; in the second trial, we performed *Salmonella* plate counting daily, from day 0 to day 12 of incubation (Fig. 1).

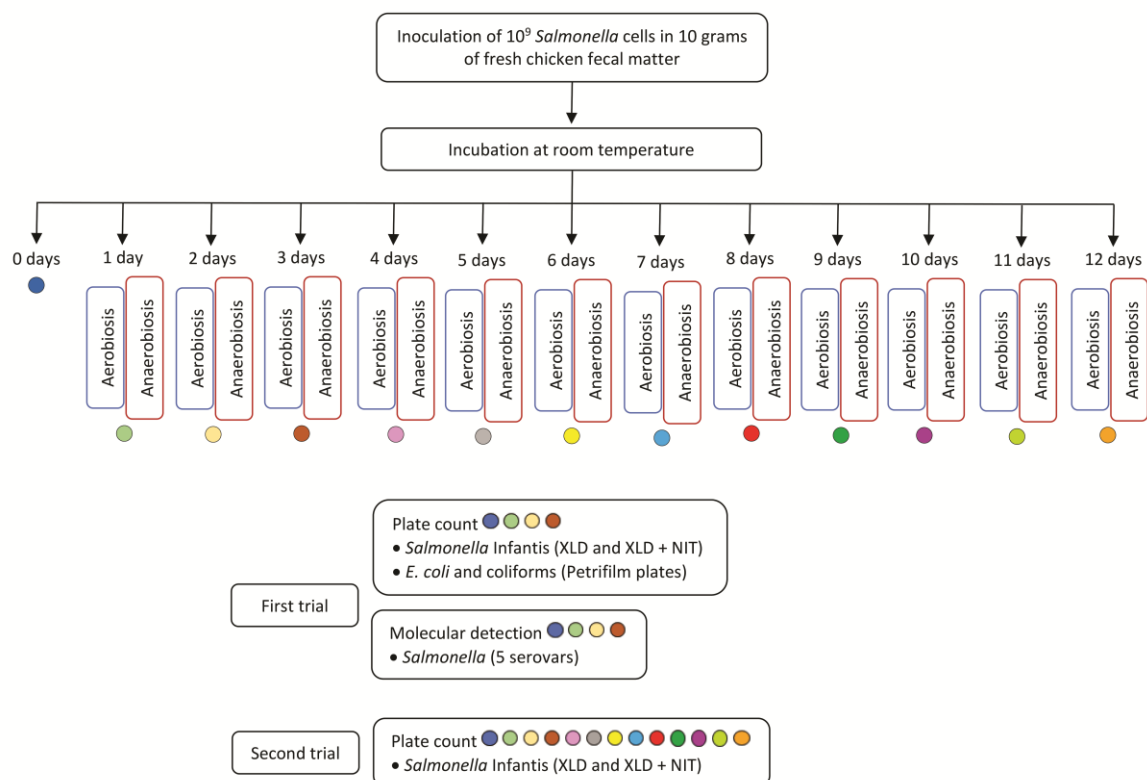


Fig. 1. Scheme of experimental procedures. The experiments performed in trial 1 and in trial 2 are indicated. In the first trial, we determined the growth of *Salmonella* by plate counting in XLD and XLD with nitrofurantoin (NIT), and by molecular detection after 0, 24, 48 and 72 h of incubation; in the second trial, we performed *Salmonella* plate counting daily, from day 0 to day 12 of incubation.

In the first trial, *Salmonella* Infantis inoculated in chicken faecal matter multiplied in both aerobic and anaerobic conditions; however, the aerobic growth was significantly higher than the anaerobic growth at 48 h ($P = 1.28 \times 10^{-4}$) and 72 h ($P = 2.94 \times 10^{-4}$). Similarly, endogenous *E. coli* growth reached its peak after 48 h, predominantly in aerobiosis ($P = 1.92 \times 10^{-2}$) and

from then on, its growth rate decreased (Fig. 2, Figs. S1-S4). The growth curve of total endogenous coliforms was similar to that of *E. coli*, with a peak in aerobiosis at 48 h ($P = 1.30 \times 10^{-2}$), but their counts were higher (Fig. S5).

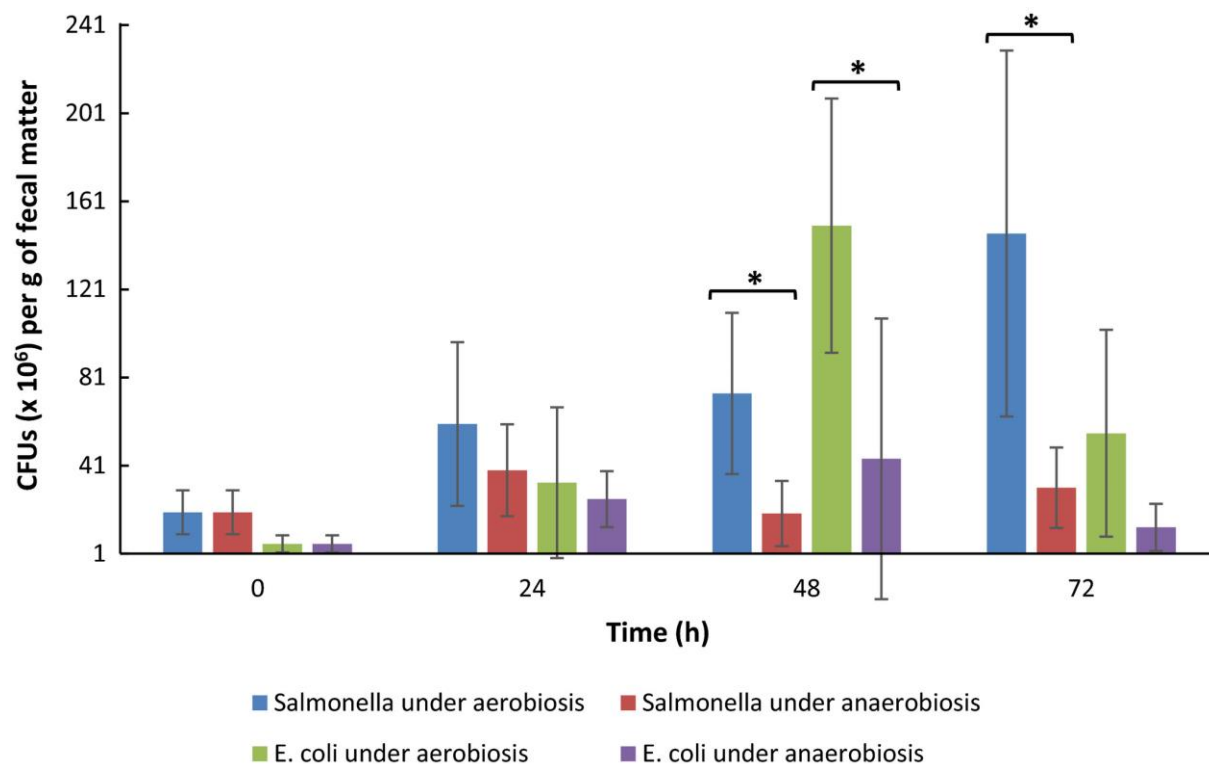


Fig. 2. Growth of *Salmonella Infantis* and endogenous *E. coli* in chicken faecal matter, under aerobic and anaerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg L⁻¹) (we took advantage of the *Salmonella* strain's resistance to nitrofurantoin to facilitate *Salmonella* colony count), and *E. coli* was counted in 3M™ Petrifilm E. coli/Coliform Count Plates. Data shown are means \pm SD. Asterisks indicate a statistically significant difference (t-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted (replicate counts) is represented by n . For *Salmonella* 0 h, 72 h of aerobiosis and 48 h of anaerobiosis $n = 14$; for 24 h of aerobiosis $n = 15$; for 48 h of aerobiosis $n = 6$; for 24 h of anaerobiosis $n = 16$; and for 72 h of anaerobiosis $n = 10$. For *E. coli* 0 h, 24 h of anaerobiosis and 72 h of anaerobiosis $n = 6$; for 24 h of aerobiosis $n = 7$, for 48 and 72 h of aerobiosis $n = 4$; and for 48 h of anaerobiosis $n = 8$. These experiments were performed twice and corresponded to the first trial.

Escherichia coli had the highest specific growth rate (μ) during the second day in aerobiosis ($P = 8.14 \times 10^{-8}$), decreasing in the following 24 h; *Salmonella* started fast growth at 24 h and presented significantly higher values of μ in aerobiosis than in anaerobiosis at all time intervals (for Δt_1 , Δt_2 , and Δt_3 , $P = 7.49 \times 10^{-5}$, 6.93×10^{-7} and 9.73×10^{-3} , respectively). Likewise,

endogenous coliforms presented higher μ values in aerobiosis than in anaerobiosis after 48 hours ($P = 1.83 \times 10^{-2}$) (Fig. 3).

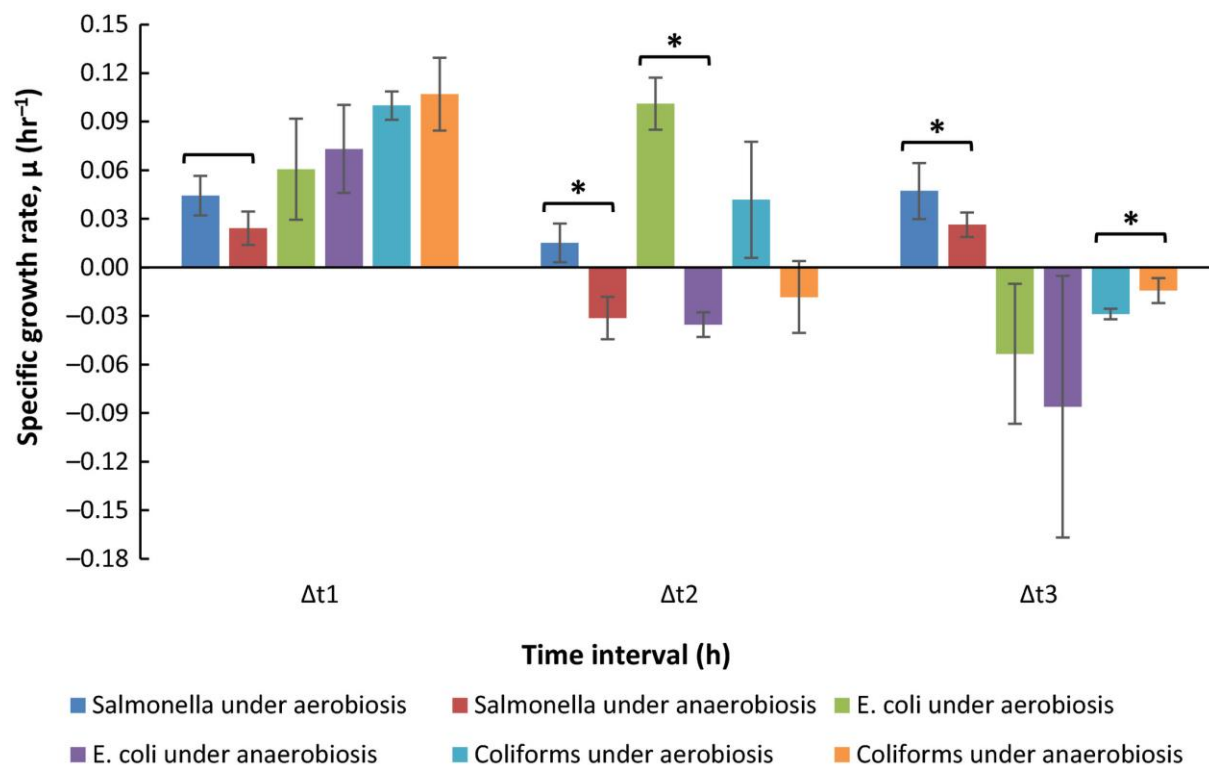


Fig. 3. Specific growth rate for *Salmonella* *Infantis*, endogenous *E. coli* and total coliforms, under aerobic and anaerobic conditions. Specific growth rate, μ , was calculated with the formula: $\mu = \frac{2.3 \log \left(\frac{N}{N_0} \right)}{\Delta t}$, where N is the final population after a time interval of incubation, Δt , and N_0 is the initial population. The incubation times were: $t_1 = 0$ h, $t_2 = 24$ h, $t_3 = 48$ h and $t_4 = 72$ h. And the intervals were: $\Delta t_1 = t_2 - t_1$, $\Delta t_2 = t_3 - t_2$, and $\Delta t_3 = t_4 - t_3$. Data shown are means \pm SD. Asterisks indicate a statistically significant difference (t -test, $P < 0.05$) between aerobic and anaerobic conditions. The number of Petri dishes counted is represented by n . For *Salmonella* Δt_1 aerobiosis and anaerobiosis, and Δt_2 anaerobiosis $n = 14$; for Δt_2 and Δt_3 aerobiosis $n = 6$; for Δt_3 anaerobiosis $n = 8$. For *E. coli* Δt_1 aerobiosis and anaerobiosis, Δt_2 anaerobiosis and Δt_3 anaerobiosis $n = 6$; for Δt_2 and Δt_3 aerobiosis $n = 4$. For total coliforms Δt_1 , Δt_2 and Δt_3 aerobiosis, and for Δt_1 anaerobiosis $n = 4$; for Δt_2 and Δt_3 anaerobiosis $n = 3$. These data correspond to the first trial.

To determine whether the above growth pattern could be applied to other *Salmonella* serovars, in the first trial we run isothermal amplification 3M™ Molecular Detection Assay 2 - *Salmonella* (MDA2SAL) at different incubation times (under aerobiosis and anaerobiosis) with 5 *Salmonella* strains (belonging to different serovars) inoculated in chicken faecal matter. The molecular assay was performed daily until day 3 after incubation (0 to 72 h). For serovars

Infantis, Heidelberg, Brandenburg, and Stanley the growth peak in aerobiosis was observed at 72 h ($P = 1.19 \times 10^{-3}$), while serovar Dublin growth peak occurred at 48 hours (Fig. 4, Fig. S6).

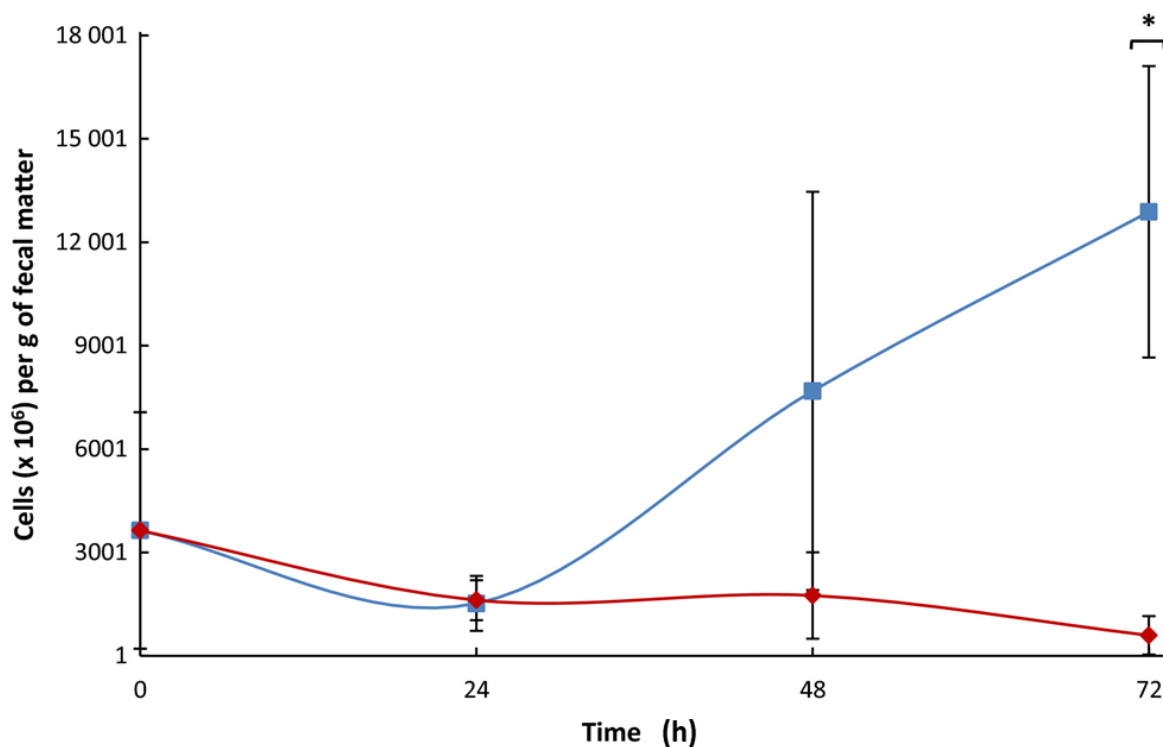


Fig. 4. Growth curves of *Salmonella* serovars: Infantis, Heidelberg, Brandenburg, and Stanley. Curves were obtained by 3M™ Molecular Detection Assay 2 - *Salmonella* (MDA2SAL). The blue line corresponds to the growth under aerobic conditions and the red one, to the growth under anaerobic conditions. Data shown are means \pm SD. Asterisk indicates a statistically significant difference (t -test, $P < 0.05$) between aerobic and anaerobic growth. The number of independent readings is represented by n ; for all data points $n = 4$. The experiment was performed once and corresponded to the first trial.

In a subsequent experiment (trial 2), no colonies of *Salmonella* in XLD or XLD with NIT were observed in aerobiosis between days 2 and 6 of incubation, probably because of a massive growth of lactose-fermenting bacteria (yellow colonies) corresponding to the commensal *Enterobacteriaceae*. Increasing *Salmonella* counts were detected on day 7 and reached a peak on day 9 (1.8×10^8 cells per g of faecal matter) (Fig. S7), which coincided with a reduction in the number of lactose fermenting bacteria colonies. On days 10 to 12, *Salmonella* growth was not detected, but lactose fermenters kept on growing, and glucuronidase reaction indicated that 94% of them were *E. coli*. We suspect that the massive growth of lactose-fermenting bacteria

was due to a different diet used in chickens during the second trial (Shang *et al.*, 2018). In anaerobiosis, we observed no growth of *Salmonella* or lactose fermenting bacteria from day 1 to 12.

Growth rates of *Salmonella* and *E. coli*, at different incubation time intervals, suggested a negative correlation which may indicate antagonism between these two bacterial genera (Fig. 3). We posit that *E. coli*'s initial massive replication may limit the availability of oxygen for *Salmonella* growth; once *E. coli* growth begins to decrease, *Salmonella* grows faster. Competition between these two bacterial genera has been described previously in the gut (Barrow *et al.*, 2015; Velazquez *et al.*, 2019) and in ready-to-eat and fresh foods, to such an extent that some authors consider that *E. coli* may not be a good indicator of *Salmonella* (Gómez-Aldapa *et al.*, 2013).

To investigate whether there was antagonism between *Salmonella* and *E. coli* in faeces, we inoculated equal concentrations (10^9 cells) of *S. Infantis* and an *E. coli* (isolated from chicken) in 10 g of sterile chicken faecal matter; inoculated samples were incubated in aerobiosis and anaerobiosis for 6 days. We observed that aerobic *E. coli* growth from day 3th to 6th was significantly higher than *Salmonella*'s (*P* values days 3 to 6 were: 1.20×10^{-5} , 1.86×10^{-2} , 1.54×10^{-6} and 5.09×10^{-5} , respectively) (Fig. S8), which suggests some level of competition between these two bacteria. This finding is in agreement with previous reports (Shang *et al.*, 2018). There were two differences between the results of the experiments in fresh faecal matter and sterilized faecal matter: (i) the interference of *E. coli* growth occurred later in sterile faecal matter (Fig. 2, Fig. S8); and (ii) there was no difference between growth under aerobic or anaerobic conditions, except for *Salmonella* on day 5 (Fig. S9). These differences may be due to physical and chemical modifications of the faecal matter by heat sterilization; autoclaved faecal matter was drier and harder probably due to dehydration and starch gelatinization

(Weurding *et al.*, 2001). Additionally, lower water activity may protect *Salmonella* (Santos *et al.*, 2005).

To ascertain whether the aerobic or anaerobic environments are determining factors in the growth of *Salmonella* and *E. coli* in chicken faecal matter we inoculated fresh faecal matter with *Lactobacillus reuteri* strain LrRR (López *et al.*, 2019), an anaerobic bacterium (Kandler *et al.*, 1980; Ianniello *et al.*, 2015), and our results showed that the growth of LrRR was significantly higher in anaerobiosis on days 2 and 3 ($P = 4.48 \times 10^{-3}$ and 6.86×10^{-5} , respectively) (Fig. S10), which is an additional evidence that the presence or absence of oxygen in the environment is a factor that determines the differential growth of *Salmonella* and *E. coli* in fresh chicken faeces. On day 6, we observed that LrRR growth in aerobiosis and anaerobiosis produced the same numbers of colonies; we speculate that aerotolerant mutant bacteria may have been selected during the incubation period, a phenomenon described previously in *Lactobacillus* (Ianniello *et al.*, 2015).

Our results indicate that *Salmonella* and other *Enterobacteriaceae* multiply massively and aerobically in fresh chicken faecal matter; in fact, faecal matter incubated under aerobic conditions has more *Salmonella* (on average 10 times more) than freshly released faeces. Our results show clear evidence that the faecal matter is a transient but very important component of the *Enterobacteriaceae* life cycle, where enterobacterial population expands (Russell and Jarvis, 2001; Vasco *et al.*, 2015; Barrera *et al.*, 2018) increasing the chances of reaching other hosts.

Previous studies have shown that *E. coli* has a negative growth rate outside the host, with a short half-life (1 day in water, 1.5 days in sediment and 3 days in soil) (Winfield and Groisman, 2003); however, we have found that as long as it remains in faecal matter, *E. coli* continues to grow up to 12 days after being excreted in the environment (intermediate habitat) (Barrera *et al.*, 2018). Also, it has been estimated that the doubling time of *E. coli* in its primary habitat

(the intestine of warm-blooded animals) is 2 days (Winfield and Groisman, 2003), and our results indicate that its doubling time in the intermediate habitat during the first two days is less than 24 hours (Fig. 2, Fig. S1). Our findings disagree with the notion that these bacteria enter a viable but not culturable status when excreted from the host (Winfield and Groisman, 2003). Additional studies are needed due to the relevance of this issue in public health.

Microbiologists have struggled to explain why bacteria adapted to the anaerobic intestinal milieu possess energetically costly machinery to use oxygen (Govantes *et al.*, 2000). Further, it has been shown that aerobic respiration is not important for *Salmonella* intestinal colonization (Barrow *et al.*, 2015). We hypothesize that the reason for this apparent evolutionary mystery may be related to the enterobacterial ability to grow in faecal matter under aerobic conditions. *Enterobacteriaceae* are facultative anaerobe which can synthesize ATP by different enzymatic pathways, depending on the external concentration of O₂ and the redox changes in the environment. When O₂ is available, the bacteria obtain energy by aerobic respiration, with O₂ being the final acceptor of electrons. In shortage of O₂, these bacteria generate ATP by one of the following mechanisms: (i) synthesis of terminal oxidases that allow the bacteria to take advantage of traces of O₂; (ii) use of other inorganic molecules (such as NO₃⁻ and S₄O₆²⁻) as final electron acceptors (Yamamoto and Droffner, 1985; Bueno *et al.*, 2012; Rivera *et al.*, 2013); and (iii) use of organic compounds as donors and acceptors (Madigan *et al.*, 2012). However, aerobic respiration produces much better performance in terms of ATP molecules per substrate molecule (Madigan *et al.*, 2012).

Salmonella is responsible for hospitalizations and deaths worldwide (Omer *et al.*, 2018; EFSA and ECDC, 2019) due to outbreaks associated not only with animal products but also with vegetables (Gunel *et al.*, 2015; Omer *et al.*, 2018). The presence of *Salmonella* in produce is associated with unintended environmental faecal contamination and the use of untreated manure as fertilizer (Fletcher *et al.*, 2013). Our results have critical implications in waste

management, contribute to select more efficient ways of treating manure through composting (Singh *et al.*, 2012; Román *et al.*, 2015) and suggest the need of anaerobic treatments for animal waste.

The loose consistency of avian faeces allows the entry of air and this phenomenon may contribute to the proficiency of these animals to spread *Salmonella*. Similarly, loose stools caused by *Salmonella* infection, may favor the growth of this bacterium in faecal matter from animals with different faecal texture.

The inconsistencies found in this study are probably due to the complex composition of faecal matter (food substrates and microbiota). Another limitation was the abundant growth of accompanying bacteria (lactose fermenters) that made it difficult the detection of *Salmonella* in XLD.

This type of studies is important because it helps to understand better the physiology of *Salmonella* and other members of the *Enterobacteriaceae* family. We addressed a neglected but crucial characteristic of *Salmonella* life cycle which may have an impact in public health.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary Material (Chapter 2)

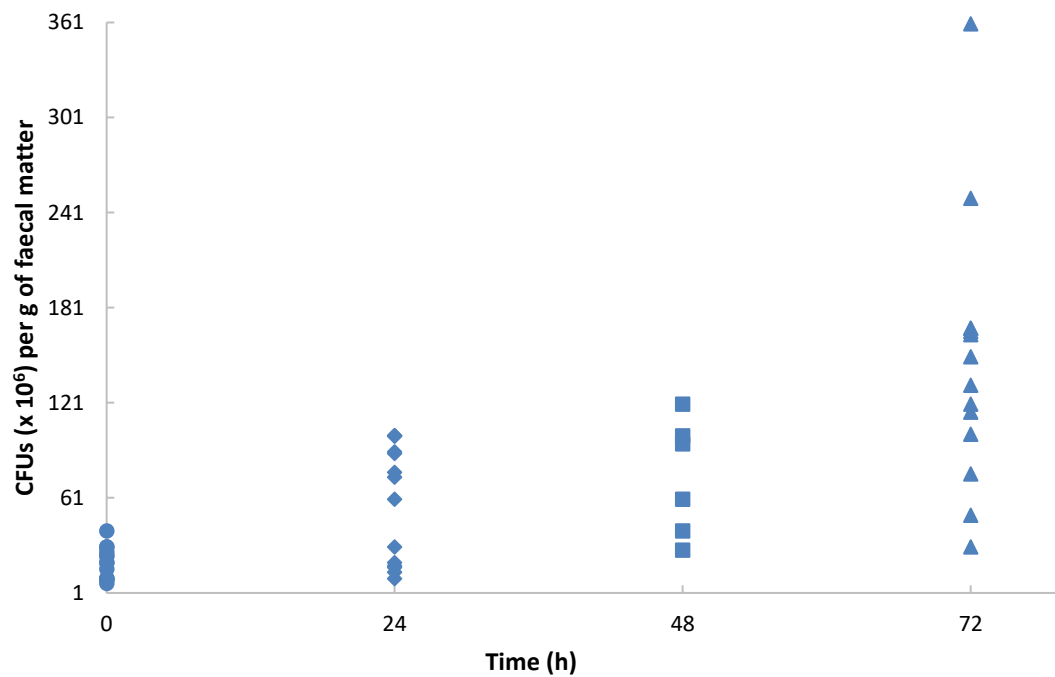


Fig. S1. Data points of the growth of *Salmonella Infantis* inoculated in chicken faecal matter, under aerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg/L). The number of Petri dishes counted is represented by n ; for 0 and 72 h $n = 14$, for 24 h $n = 15$, and for 48 h $n = 6$. The experiment was performed twice and corresponded to the first trial.

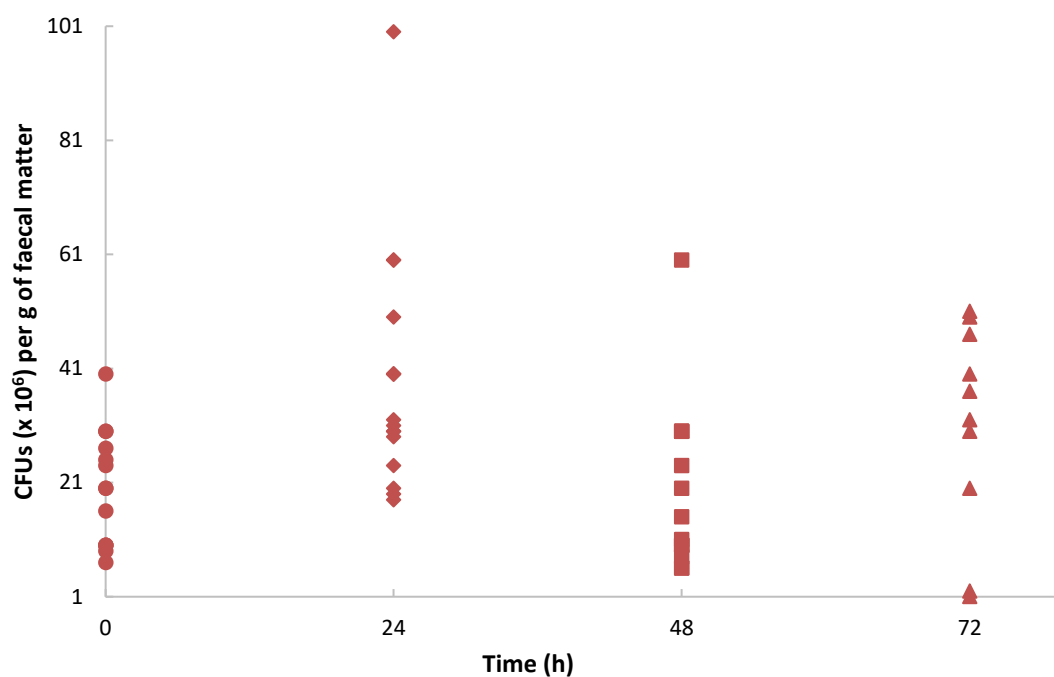


Fig. S2. Data points of the growth of *Salmonella Infantis* inoculated in chicken faecal matter, under anaerobic conditions. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg/L). The number of Petri dishes counted is represented by n ; for 0 and 48 h $n = 14$, for 24 h $n = 16$, and for 72 h $n = 10$. The experiment was performed twice and corresponded to the first trial.

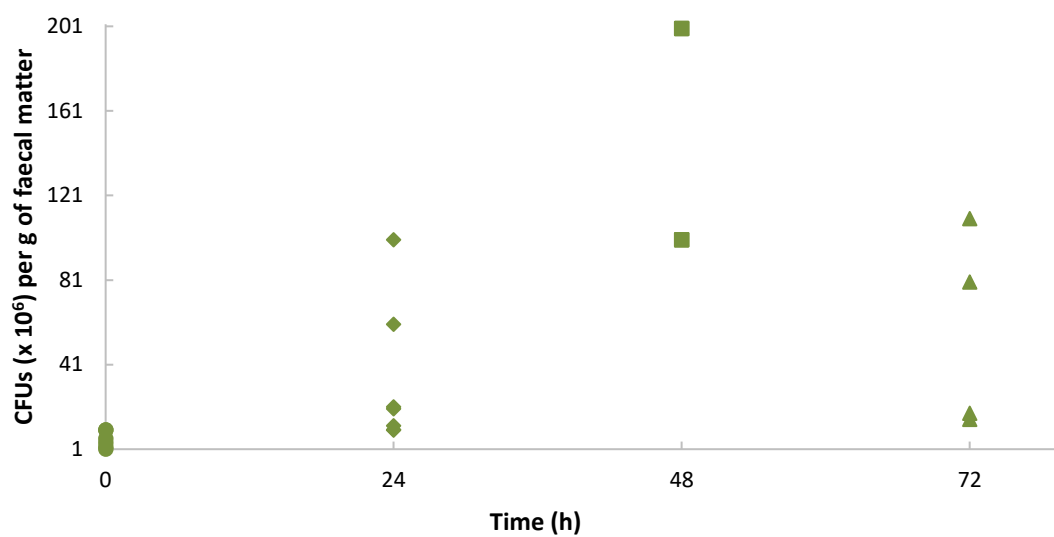


Fig. S3. Data points of the growth of endogenous *E. coli* in chicken faecal matter, under aerobic conditions. *E. coli* was counted in 3M™ Petrifilm *E. coli*/Coliform Count Plates. The number of Petri dishes counted is represented by *n*; for 0 h *n* = 6, for 24 h *n* = 7, for 48 and 72 h *n* = 4. The experiment was performed twice and corresponded to the first trial.

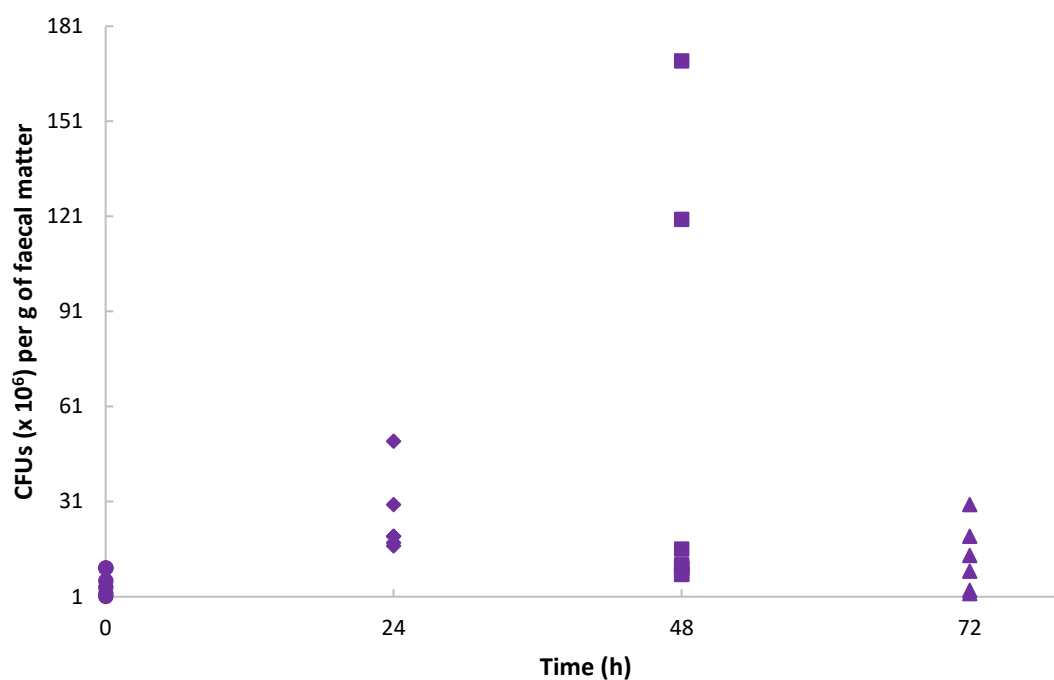


Fig. S4. Data points of the growth of endogenous *E. coli* in chicken faecal matter, under anaerobic conditions. *E. coli* was counted in 3M™ Petrifilm *E. coli*/Coliform Count Plates. The number of Petri dishes counted is represented by n ; for 0, 24 and 72 h $n = 6$, for 48 h $n = 8$. The experiment was performed twice and corresponded to the first trial.

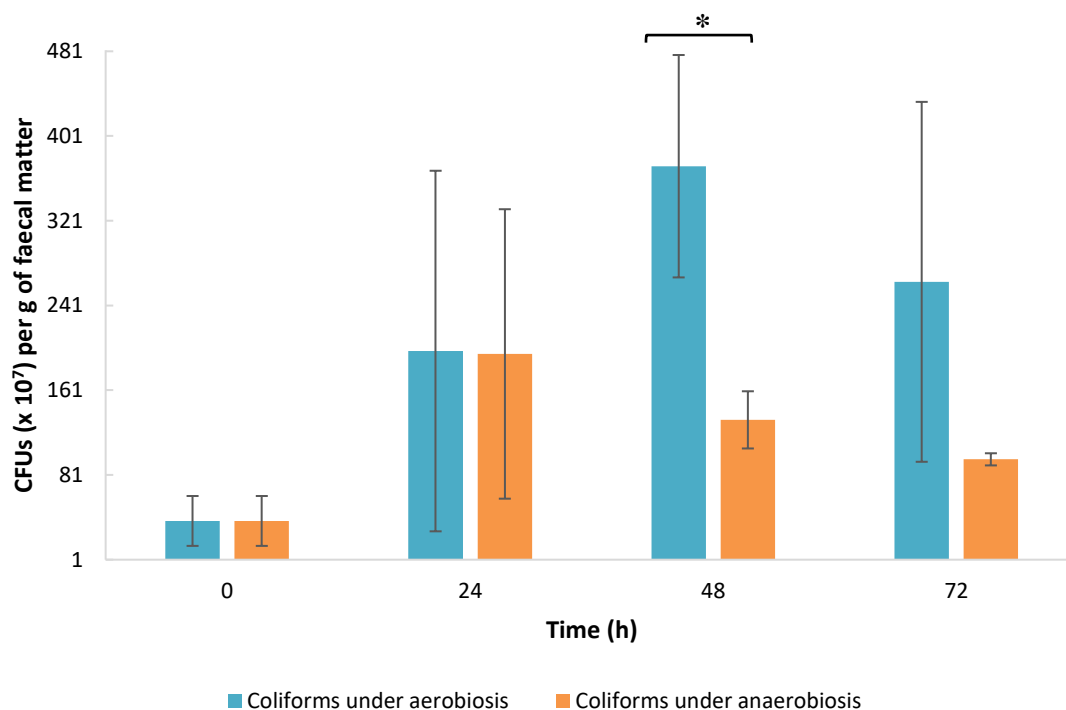


Fig. S5. Growth of endogenous total coliforms in chicken faecal matter, under aerobic and anaerobic conditions. The number of total coliforms corresponded to the sum of the red and blue colonies with gas in 3M™ Petrifilm E. coli/Coliform Count Plates incubated 24 and 48 h. Data shown are means \pm SD. Asterisk indicates statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*; for 0 h $n = 8$; for 24 h aerobiosis and anaerobiosis, 48 h aerobiosis and 72 h anaerobiosis $n = 4$; for 72 h aerobiosis $n = 6$; and for 48 h anaerobiosis $n = 3$. The experiment was performed twice and corresponded to the first trial.

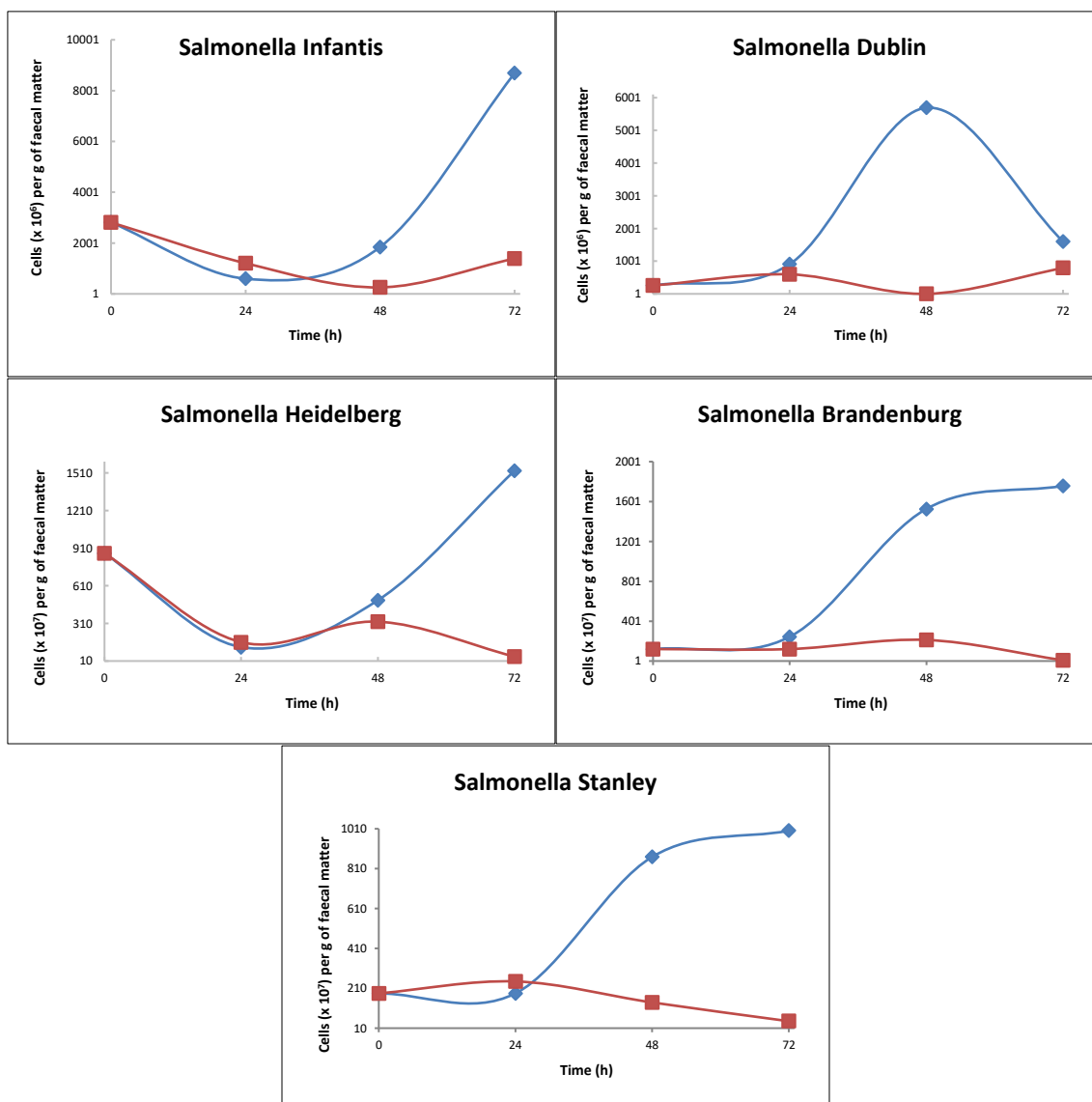


Fig. S6. Individual growth curves of *Salmonella* serovars. These curves were obtained by 3M™ Molecular Detection Assay 2 - *Salmonella* (MDA2SAL). The blue lines correspond to the growth under aerobic conditions and red ones, under anaerobic conditions. The number of independent readings is represented by n ; for all data points $n = 1$. The experiment was performed once and corresponded to the first trial.

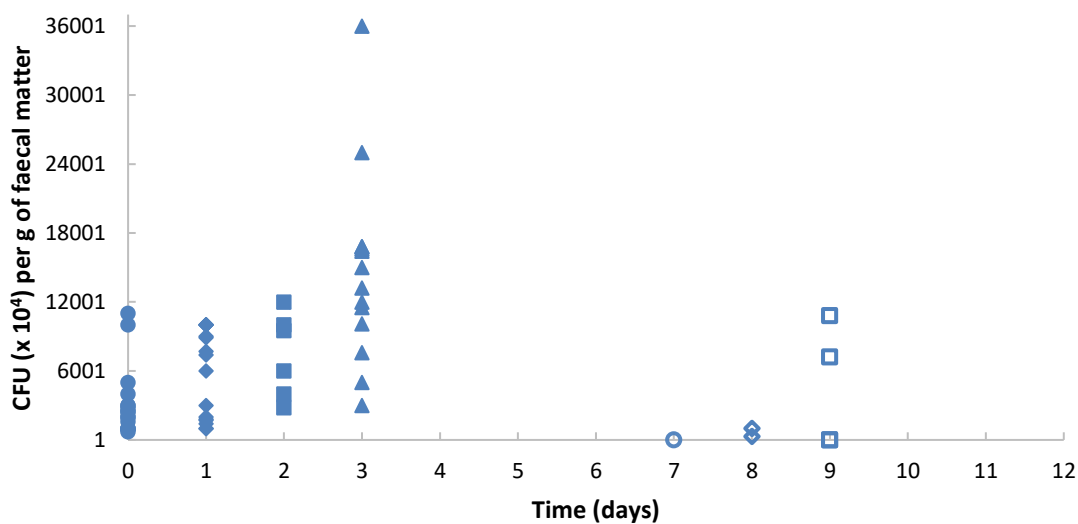


Fig. S7. Data points of the growth of *Salmonella Infantis* inoculated in chicken faecal matter, under aerobic conditions, days 0 to 12. Typical *Salmonella* colonies were counted in XLD and XLD with NIT (12 mg/L). This graph considers the results of the first trial (2 repetitions) and the second trial (1 repetition). The number of Petri dishes counted is represented by n . For 0 days $n = 17$, for 1 day $n = 16$, for 2 and 9 days $n = 6$, for 3 days $n = 14$, for 7 days $n = 1$ and for 8 days $n = 2$.

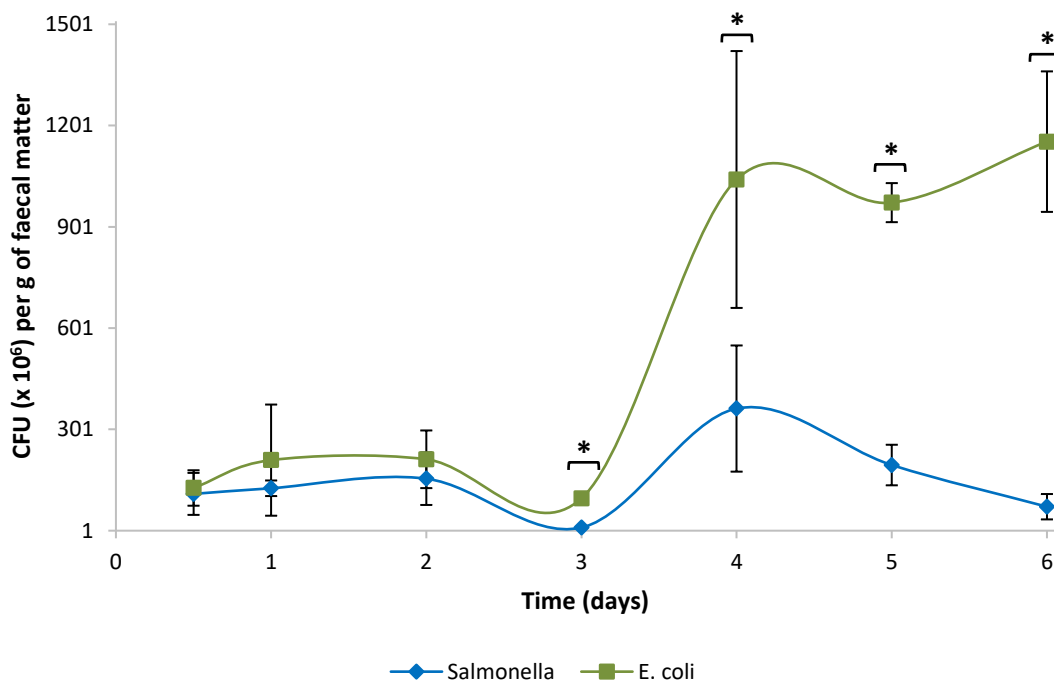


Fig. S8. Growth curves of *Salmonella* Infantis and *E. coli* inoculated in sterile faecal matter, under aerobic conditions. Colonies were counted in MKL. Data shown are means \pm SD. Asterisks indicate statistically significant difference (*t*-test, $P < 0.05$) between the number of *Salmonella* and *E. coli*. The number of Petri dishes counted is represented by *n*. For *Salmonella* $n = 4$, except on days 1 ($n = 3$) and 2 ($n = 2$). For *E. coli* $n = 4$, except on day 1 ($n = 3$). The experiment was performed once.

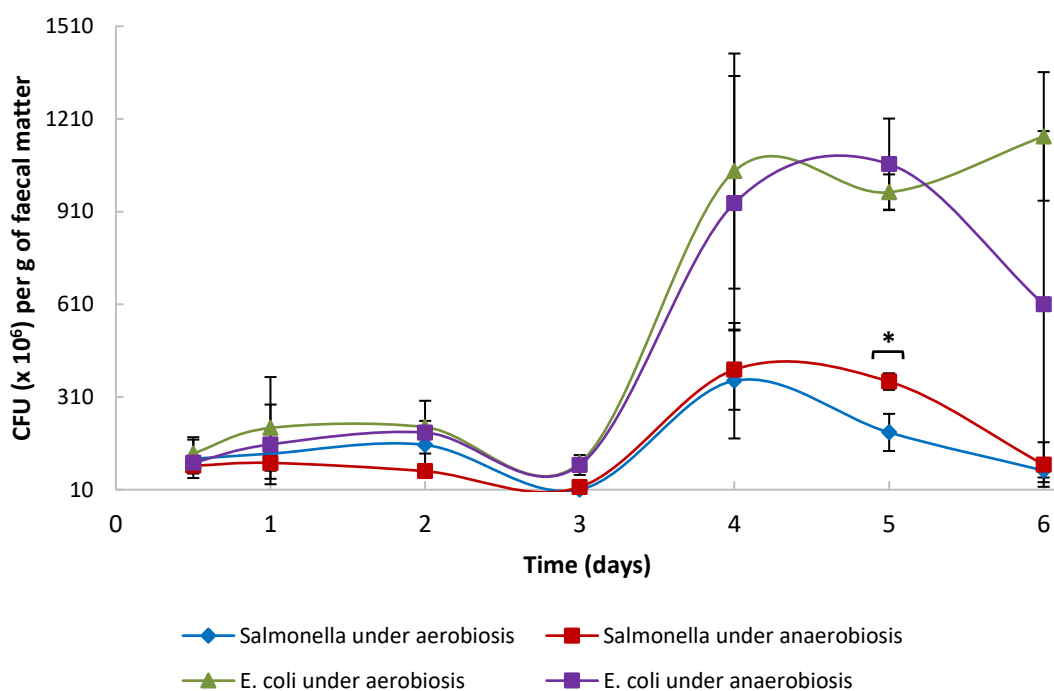


Fig. S9. Growth curves of *Salmonella* Infantis and *E. coli* inoculated in sterile faecal matter, under aerobic and anaerobic conditions. Colonies were counted in MKL. Data shown are means \pm SD. Asterisk indicates statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*. For *Salmonella* $n = 4$, except on day 1 aerobiosis and anaerobiosis, and day 3 anaerobiosis ($n = 3$), day 2 aerobiosis and anaerobiosis ($n = 2$) and day 6 anaerobiosis ($n = 8$). For *E. coli* $n = 4$, except on day 1 aerobiosis and anaerobiosis ($n = 3$), day 2 anaerobiosis ($n = 2$) and day 6 anaerobiosis ($n = 8$). The experiment was performed once.

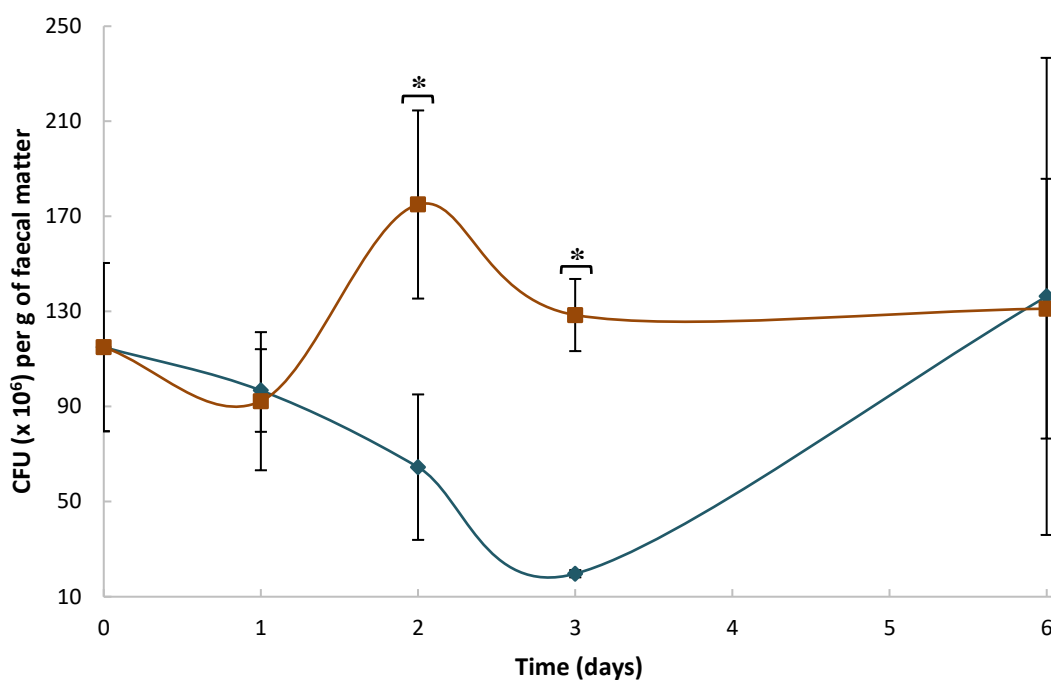


Fig. S10. Growth curves of *Lactobacillus reuteri* rifampicin resistant in chicken faecal matter, under aerobic and anaerobic conditions. Colonies were counted in MRS agar + Rifampicin (100 $\mu\text{g/mL}$). The brown line corresponds to the growth under anaerobiosis and the blue one, under aerobiosis. Data shown are means \pm SD. Asterisks indicate statistically significant difference (*t*-test, $P < 0.05$) between aerobic and anaerobic growth. The number of Petri dishes counted is represented by *n*. For the data points $n = 4$, except for day 3 aerobiosis ($n = 3$) and day 6 ($n = 6$). The experiment was performed once.

Data S1. Experimental Procedures

Overall approach

Five fresh *Salmonella* (isolates from poultry) were inoculated in chicken faecal matter and incubated at room temperature under aerobic and anaerobic conditions. *Salmonella* colonies number was determined by culture and by the loop-mediated isothermal amplification 3M™ Molecular Detection Assay 2 - *Salmonella* (MDA2SAL) (Fig. 1).

The *Salmonella* isolates were identified as *Salmonella enterica* serovars Infantis, Dublin, Heidelberg, Brandenburg and Stanley utilizing a multiplex PCR (Kim *et al.*, 2006). A *Salmonella* Infantis (strain POL 398 B) resistant to nitrofurantoin was used for plate count tests.

Salmonella inoculation in chicken faecal matter

Each *Salmonella* strain was cultured in 4 mL of Brain Heart Infusion broth, then the culture was centrifuged for 5 min at 4,000 xg, the supernatant was discarded, and the pellet was re-suspended in 500 µL of sterile saline solution. The process was repeated once to eliminate remnants of culture medium and the resulting suspension was used to inoculate chicken faeces. Before inoculation, the number of *Salmonella* cells per mL of suspension was determined by counting in a Petroff-Hausser chamber.

In a first trial, faecal material was obtained from 3 2-week old broiler chickens *Salmonella* free (analyzed using the loop-mediated isothermal amplification method 3M™ MDA2SAL). The elapsed time between excretion and use in the experiments varied from 0 to 16 h, depending on the amount of faecal matter needed. To reduce the stool aging effect on bacterial growth, all the faecal material collected for each experiment was pooled and mixed.

Faecal matter pooled was split into 10 g aliquots and placed in 35 Petri dishes. In each set of 7 dishes, it was inoculated 100 µL of suspension (10^9 cells per mL) of a *Salmonella* serovar. The

faecal matter of one plate in each set was used for counting at 0 h (i.e., immediately after blending); of the remaining dishes, half were incubated in aerobiosis and a half in anaerobiosis, at room temperature, for 24, 48 and 72 h (Vasco *et al.*, 2015). The anaerobic atmosphere was created using BD GasPak™ EZ Anaerobe Gas Generating Pouch System with Indicator.

In a subsequent experiment (trial 2), the procedure was as indicated above, but the incubation was carried out up to 12 days (faecal material for this experiment was obtained from another 3 2-week old broiler chickens *Salmonella* free; in this trial, chickens received different diet).

Colony counts

In the first trial (three days), two dishes containing faecal matter inoculated with *S. Infantis* incubated under aerobic and anaerobic conditions was subjected to colony count in culture media for *Salmonella*, *E. coli*, and coliforms. Briefly, after 24-h incubation, we diluted the contents of each Petri dish (up to 10^{-8} in buffered peptone water, BPW), and plated dilutions 10^{-6} , 10^{-7} and 10^{-8} onto Xylose Lysine Deoxycholate Agar (XLD) and XLD with nitrofurantoin (NIT) (12 mg/L) (Sandegren *et al.*, 2008) (we took advantage of the *Salmonella* strain's resistance to nitrofurantoin to facilitate *Salmonella* colony count). Typical *Salmonella* colonies were counted in XLD and XLD with NIT to ensure that the fitness of the bacteria was not affected in the medium with an antibiotic. *Salmonella* counts were similar in both media. These three dilutions were also inoculated onto 3M™ Petrifilm *E. coli*/Coliform Count Plates and incubated for 24 and 48 h at 37 °C, to determine the number of endogenous *E. coli* and coliforms. We counted blue colonies with gas (*E. coli*) and red colonies with gas (other coliforms). The number of total coliforms corresponded to the sum of the blue and red colonies associated with gas. For each treatment, it was included and analyzed a faecal sample without *Salmonella* as a control.

In the second trial, *S. Infantis* colonies were counted in XLD and XLD with NIT up to 12th day (dilutions 10^{-4} to 10^{-8}). Yellow colonies of lactose fermenting bacteria that grew in XLD with NIT were inoculated in Chromocult Agar to determine *E. coli* and coliform counts.

Calculation of specific growth rate, μ

The specific growth rate (μ) was calculated using the formula:

$$\mu = \frac{2.3 \log \left(\frac{N}{N_0} \right)}{\Delta t}$$

where N is the final population after a time interval of incubation, Δt , and N_0 is the initial population (Maier, 2009; Montville *et al.*, 2012).

Molecular detection

The number of *Salmonella* cells in the chicken faecal matter at different time points was also determined by the loop-mediated isothermal amplification method 3M™ MDA2SAL. A calibration curve (Gandelman *et al.*, 2010) was carried out using bacteria suspended in stool sample; bacterial cell suspensions (10^{-2} to 10^{-10}) were prepared in a 1:10 dilution of faecal matter in BPW. Additionally, for each trial, we run a suspension with a known number of cells (Petroff-Hausser chamber) of the strain analyzed. A 10^9 cells/mL suspension of each of the 5 different *Salmonella* serovars (Infantis, Dublin, Heidelberg, Brandenburg, and Stanley) inoculated in fresh faecal matter was monitored during days 0, 1, 2 and 3. We used a regression equation to calculate the concentration of *Salmonella* cells at each time point (Gandelman *et al.*, 2010).

Inoculation of Salmonella and E. coli in sterile faecal matter

Similar concentrations (10^9 cells) of *E. coli* (isolated from chicken faeces) and *S. Infantis* were inoculated in sterile (autoclaved) chicken faecal matter and incubated at room temperature in

aerobiosis and anaerobiosis as described above and bacterial growth was monitored daily after (12 hours to 6 days); colonies were counted in MKL.

Anaerobic growth

To test the anaerobic conditions in faecal matter, a *Lactobacillus reuteri* rifampicin-resistant strain LrRR (López *et al.*, 2019) was used. A suspension of LrRR cells (10^9 cells/mL counted in Petroff-Hausser chamber) was inoculated in chicken faecal matter as was indicated above and incubated at room temperature for 0, 1, 2, 3 and 6 days under aerobic and anaerobic conditions. Dilutions were prepared in peptone water 0.1%, inoculated onto Man Rogosa Sharpe agar (MRS) with rifampicin (100 µg/mL) (Miller, 1992) and incubated at 37 °C for 72 h under microaerophilic conditions. Typical colonies of LrRR were counted and analyzed by Gram stain, catalase and oxidase tests (López *et al.*, 2019).

Statistic analysis

Our null hypothesis (H_0) was that there is no significant difference in the growth of *Salmonella* in chicken faecal matter under aerobic and anaerobic conditions, and the alternative hypothesis (H_1), that there is a significant difference. To evaluate them, we performed the *t*-test (two-tailed) for two samples assuming equal variances, with a significance level of 0.05.

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CHAPTER 3

Use of bacteriophages to control *Salmonella* in poultry operations

Abstract

Antimicrobial resistance is a growing global public health problem; for this reason, the World Health Organization has recommended against the use of antibiotics as growth promoters in animal husbandry. Antibiotics are also used routinely to control *Salmonella* in chickens which are frequent carriers of this pathogen. In Latin America, the most frequent serotypes in chicken products are Enteritidis and Infantis, evidencing numerous reports of multidrug-resistant isolates. To diminish the use of antibiotics, the poultry industry seeks alternatives to reduce nontyphoidal *Salmonella* in chicken meat and one option is the use of bacteriophages. In this context, the objectives of this project were to isolate lytic *Salmonella* bacteriophages from environmental samples, and to define the best phage application method to eliminate the pathogen in chickens from a commercial farm. *Salmonella* bacteriophages were isolated from river water and samples related to poultry production (chicken bedding samples, and water from different points of poultry slaughter and processing plants). Then, the most lytic candidates were evaluated for on-farm application. Oral phage administration (with drinking water) in combination with bedding spray of phages eliminated *Salmonella* from chicken intestine for 10 days. Our results suggest that the use of bacteriophages to control *Salmonella* in poultry industry is a viable and safe alternative to the use of antibiotics.

Introduction

Antimicrobial resistance (AMR) is an increasing global public health problem; for this reason, the World Health Organization rejects the use of antibiotics as growth promoters in animal

husbandry, in order to preserve the effectiveness of important antibiotics in human medicine. This decision was made considering that up to 80% of the consumption of antibiotics of medical importance was destined for animal husbandry (WHO, 2021). Consistent with this concern, the poultry industry seeks alternatives to combat bacteria that are pathogenic for chickens and humans (Wessels, Rip & Gouws, 2021).

Foodborne salmonellosis outbreaks are a global public health problem that is exacerbated by AMR. Therefore, alternative methods are being used in poultry husbandry to reduce the presence of nontyphoidal *Salmonella* (NTS), including the use of compounds with chlorine and organic acids, although their disadvantage is that they affect the sensory properties of the meat (Wessels *et al.*, 2021). An interesting option is bacteriophages, viruses that adhere to specific receptors on the bacterial cell wall, so they are considered non-pathogenic for humans and Generally Recognized As Safe (GRAS) for consumption. For the successful implementation of its use, it is necessary to take into account factors such as local legislation and application volumes and concentrations (Hagens & Loessner, 2010; CDC, 2021; Wessels *et al.*, 2021).

Bacteriophages (hereinafter called phages) are classified according to their morphology, nucleic acid (ssRNA, dsRNA, ssDNA, and dsDNA), life cycle, and bacterial host (Żbikowska, Michalczyk & Dolka, 2020). Their replication cycle can be lytic (virulent phages) or lysogenic (tempered phages); and some phages have both the lytic and lysogenic cycles. In the lytic cycle, the phages replicate inside the host bacteria and release their progeny killing the host cell to infect new bacteria. In the lysogenic cycle, the genetic material of the phage is integrated into the bacterial chromosome, being vertically transmitted to the bacterial offspring (Wernicki, Nowaczek & Urban-Chmiel, 2017; Żbikowska *et al.*, 2020). The integrated phage or prophage can become activated and enter a lytic cycle under stress conditions (Wernicki *et al.*, 2017; Żbikowska *et al.*, 2020; Wessels *et al.*, 2021).

Most of the phages described, including *Salmonella* phages, belong to the order *Caudovirales* (they have a tail) that includes the families *Siphoviridae* (long non-contractile tail), *Myoviridae* (long contractile tail), and *Podoviridae* (short non-contractile tail); its size ranges between 20 and 200 nm (Dion, Oechslin & Moineau, 2020; Żbikowska *et al.*, 2020).

Lytic phages are specially sought in the food industry because temperate phages, by inserting their genome into the bacterial chromosome, could alter the phenotype of the bacterial host (lysogenic conversion), and sometimes could increase bacterial pathogenicity (Hagens & Loessner, 2010; Fillol-Salom *et al.*, 2019).

The first record of phages used to eliminate *Salmonella* in poultry dates from 1917, when D'Herelle developed a phage therapy against fowl typhoid by *Salmonella enterica* serovar Gallinarum in chickens (Wernicki *et al.*, 2017). Since then, the phage application in the poultry industry is increasingly widespread. Some examples in American countries are cited in Table 1.

However, a major limitation of phage use is that bacteria could develop resistance to phages by: (1) Preventing phage adsorption by means of blocking of phage receptors, production of extracellular matrix or production of competitive inhibitors; (2) preventing phage DNA entry into host cells, using the superinfection exclusion (Sie) systems (blocking proteins); (3) cutting phage nucleic acids by mean of restriction-modification systems or the CRISPR-Cas system; and (4) developing abortive infection systems that typically target a crucial step of phage multiplication such as replication, transcription or translation (Labrie, Samson & Moineau, 2010). Phage resistance in *S. Typhimurium* was found to be related to reduce LPS production and thus lower availability of the phage receptor for adsorption (Wang *et al.*, 2019).

In Ecuador, *Salmonella* is present in 88% of chicken carcasses, where 91% of strains are multidrug-resistant (MDR). 94% of the isolates belong to serovar Infantis (Vinueza-Burgos *et al.*, 2019). To reduce the use of antimicrobials, some poultry farms have explored the possibility

of using phages. The objectives of this project were to isolate *Salmonella* phages from environmental samples (river water and samples related to poultry production), and to define the best phage application method to eliminate *Salmonella* in chickens from a commercial farm.

Methods

Strains

Salmonella enterica serovar Typhimurium ATCC 14028, *Salmonella enterica* from poultry farms (serovars Infantis, Braenderup, and Saintpaul), and *Salmonella enterica* serovar Infantis from raw chicken meat were used to isolate phages. *Salmonella enterica* from chicken bedding (serovars Infantis, Braenderup, Saintpaul, and Albany) and from raw chicken meat (serovars Infantis, Brandenburg, Dublin, Heidelberg, Stanley, and Typhimurium), *Salmonella enterica* serovar Infantis from chicken skin and from chicken caeca, and *Salmonella enterica* serovar Amsterdam from animal feed, were used to determine the phages host range.

Escherichia coli from chicken bedding, and *Citrobacter werkmanii* and *Edwardsiella tarda* from a poultry slaughter plant were also used for phages host range tests. The bacterial cultures were stored at -80 °C in skim milk with 10% glycerol.

Media and buffers

BHI: Brain heart infusion broth (BD, France). Suspend 37 g of the medium in one liter of distilled water; mix, boil for one minute until complete dissolution, and sterilize at 121 °C for 15 minutes. **DSPB** (Decca Strength Phage Broth): Mix peptone 100 g, yeast extract 50 g, K₂HPO₄ 80 g, and distilled water 1000 mL; pH was adjusted to 7.6 after sterilizing the medium in autoclave (121 °C for 15 minutes). **Phage buffer:** 10 mM Tris-HCl (pH 7.5), 68.5 mM NaCl, 10 mM MgSO₄, 1 mM CaCl₂; an autoclave step was realized (121 °C for 15 minutes) separately for each solution and then mixed. **Semi-solid agar with Mg:** Suspend BHI + 0.7% agar + 0.2% MgSO₄ in distilled water, boil to complete dissolution, sterilize for 15 min at 121 °C, and keep

molten (in a 50 °C water bath) until use. Skim milk with glycerol: Mix 10% skim milk and 10% glycerol in distilled water, dispense 1 mL into microtubes, and sterilize for 15 min at 121 °C. Solid agar with Mg: Suspend BHI + 1.5% agar + 0.2% MgSO₄ in distilled water, boil to complete dissolution, sterilize for 15 min at 121 °C, and dispense into Petri dishes. Solid nutrient agar: Suspend BHI + 1.5% agar in distilled water, boil to complete dissolution, sterilize for 15 min at 121 °C, and dispense into Petri dishes (Benson, 1980; Piuri, 2019).

Samples for phage isolation

Phages were isolated from the following samples: Wastewater from Machángara and San Pedro rivers (located in Quito, Ecuador), chicken bedding samples, water from different points of a poultry slaughter plant (pre-chiller, chiller, scalding, gutting machine, and final poultry washing), washing water from poultry cutting and marinating machines (Fig. 1 and Table S1).

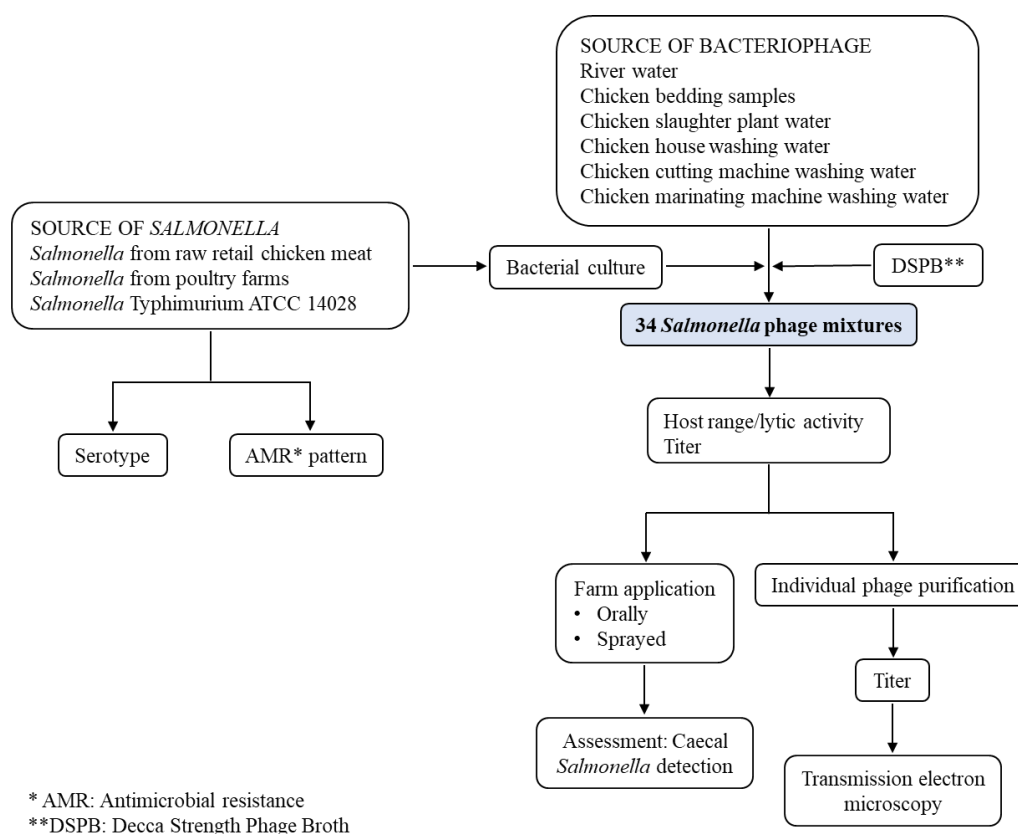


Figure 1. Scheme of the experimental procedures of phage isolation and on-farm application.

Isolation of Salmonella phages

The Benson procedure was followed for phages isolation. Briefly, 45 mL of water sample or 1:1 suspension of chicken bedding sample in sterile distilled water was mixed with 5 mL of 12-16 h *Salmonella* culture in BHI and 5 mL of DSPB and incubated at 37 °C for 24 h. Then, the suspension was centrifuged at 7000 xg for 15 minutes and filtered using a 0.45 μm membrane, retaining bacteria and only phage pass through the filter. The phage filtrate was preserved by adding chloroform (3 chloroform drops per 5 mL of culture, or 3%) (Benson, 1980).

DNA extraction from Salmonella isolates and serotyping

DNA extraction from *Salmonella* isolates was done by boiling for 10 minutes a suspension of 3-5 colonies of a pure culture from solid nutrient agar (Dashti *et al.*, 2009) in PCR water. Then, a 1/100 dilution of the DNA was made also in PCR water, and serotyping was carried out. The method described by Kim and colleagues was followed to serotype *Salmonella* isolates. Briefly, it consisted in two multiplex PCRs that discriminate 30 serotypes of *S. enterica* subsp. *enterica*. 6 genetic loci of *Salmonella enterica* serovar Typhimurium (STM1-STM6) and 4 of *Salmonella enterica* serovar Typhi (STY1-STY4) were used. Of the 30 serotypes that can be discriminated, 22 present unique amplification patterns, and 8 are grouped into 4 pairs. To resolve these pairs, 2 additional PCRs can be performed (primer sets STM7 and PT4-1; the latter based on genomes of Enteritidis and Dublin serovars) (Kim *et al.*, 2006).

Antimicrobial resistance of Salmonella isolates

Antibiogram of the *Salmonella* isolates was carried out, following the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. The antimicrobials tested were amoxicillin-clavulanic acid (AMC- 20/10 μg), ampicillin (AM-10 μg), azithromycin (AZM-15 μg), cefotaxime (CTX- 30 μg), ceftriaxone (CRO-30 μg), chloramphenicol (C-30 μg), ciprofloxacin (CIP-5 μg), gentamicin (GM-10 μg), nalidixic acid (NA-30 μg), tetracycline (TE-30 μg), streptomycin (S-

10 µg), and sulphamethoxazole-trimethoprim (SXT-23.75/1.25 µg) (Hudzicki, 2009; CLSI, 2021). The trial was performed once ($n = 1$).

Phages host range and lytic activity

Phage host range was determined by spotting the phage mixtures on a lawn of each of the 38 *Salmonella* isolates and 3 other isolates from *Enterobacteriaceae* family (*E. coli*, *C. werkmanii*, and *E. tarda*). Briefly, a logarithmic phase culture (12-16 h) of each test isolate was spread on the surface of a solid agar with Mg, 2 µL of each phage mixture was placed in a cell of a grid drawn on the Petri dish and incubated at 37 °C for 24 h. Then, the phages lytic activity was evaluated, rating their virulence level according to the following scale: 4, complete clearing; 3, total clearing with pinpoint colonies within the plaques; 2, turbidity throughout the cleared zone; 1, a few individual plaques; 0, no clearing (Huang *et al.*, 2018).

Phage titer

To determine the titer of the phage mixtures and the individual phages, the double-layer agar (DLA) technique was used. Serial dilutions (10^{-1} to 10^{-10}) of phage suspensions were made in phage buffer. 4 mL of semi-solid agar with Mg was mixed with 300 µL of *Salmonella* culture and poured on the surface of solid nutrient agar. Once the agar solidified, 2 µL of phage dilution was placed in each cell of a grid drawn in the Petri dish and incubated at 37 °C for 24 h. The phage titer, in plaque forming units (PFU) per mL, was calculated with the formula: PFU/mL = Number of lysis plaques / (Inoculum volume (mL) x Dilution) (Piuri, 2019).

Phage application in poultry farms

The most lytic phage mixture against *Salmonella* (M-115A) was used for on-farm application. In preliminary essays, the volume and the conditions of phages administration to chickens together with the drinking water were defined. In the first trial, the phages were administered orally to chickens within houses that previously tested positive for *Salmonella*. The phage administration was done with the drinking water in a 1000:0.2 water:phage ratio, after 2 h of

water supply suspension, together with the viral protector VAC-PAC PLUS[®], that stained the water blue, to control that all the chickens drink it. In the second trial, the application was carried out orally and by spraying the phages dissolved in water, using hand pumps, on the chicken bedding. On day 1, orally and by spraying, and on days 2 and 3 only by spraying (Clavijo *et al.*, 2019; Coba, 2020). To assess the phages effectiveness, on each sampling day, 25 g of a pool of caeca of 25 randomly sampled chickens was analyzed to detect *Salmonella*. These analyzes were carried out in the Bacteriology Laboratory of the Faculty of Veterinary Medicine and Zootechnics, Universidad Central del Ecuador, based on the ISO 6579 Standard.

Individual phages purification

Individual phages from a mixture were purified using the DLA technique. Briefly, 300 µL of a log phase *Salmonella* culture and 300 µL of each decimal dilution of the phage suspension (10^4 to 10^{-8}) were left in contact for 10 minutes, then, molten semi-solid agar with Mg (top agar) was added, and the mixture was poured onto a solid nutrient agar (bottom agar) surface and incubated at 37 °C for 24 hours. The following day, the number of lysis plaques was counted to calculate the phage titer and a lysis plaque was taken with a sterile toothpick, resuspended in 150 µL of phage buffer and labeled as "1st purification". The process was repeated 5 consecutive times to purify the individual phages, and the microtubes with the suspension of the lysis plaques were refrigerated (4 °C), labeled with the corresponding purification. The phage suspension resulting from the last purification was titrated (Clockie & Kropinski, 2009; Piuri, 2019).

Transmission electron microscopy

A transmission electron microscope (TEM) (Tecnai G2 Spirit Twin, FEI, Holland) operated at 80 kV was used to observe phages. Tungstophosphoric acid (PTA) purchased from Ted Pella was prepared at 0.5% (w/v) aqueous solution, adjusted pH 7. Single-droplet negative staining technique was applied on our semipurified aqueous suspension, and formvar (thermoplastic

resin) support films (300 mesh) were used (Kuo, 2007). These analyzes were carried out in the Nanomaterials Characterization Laboratory, CENCINAT, Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador. The phages size in the micrographs was evaluated using FIJI software (ImageJ 1.53f51) (Schindelin *et al.*, 2012).

Results

Salmonella phage mixtures isolation

In total, 34 phage mixtures were isolated from 7 types of samples; the *Salmonella* host isolates came from poultry farms and raw retail chicken meat, in addition to the *Salmonella* Typhimurium ATCC 14028 strain. Table 2 and Fig. 2 show how many phage mixtures were isolated from each type of sample and the origin of the host bacterium.

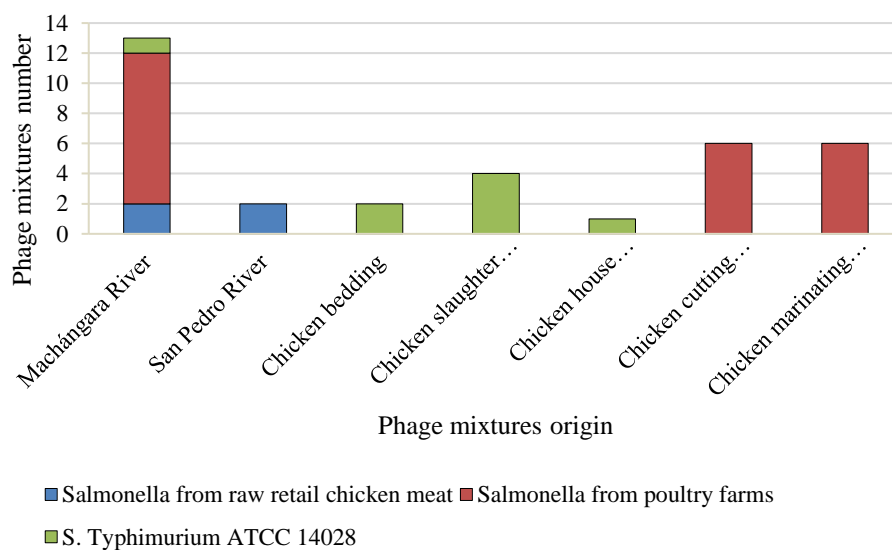


Figure 2. Origin and number of the isolated *Salmonella* phage mixtures. The X-axis shows the source of the phages. The colors of the bars indicate the origin of the host *Salmonella* isolate.

Origin and serovars of Salmonella isolates

The lytic activity of *Salmonella* phage mixtures was tested *in vitro* against 38 *Salmonella* isolates obtained from chicken caeca and skin, raw retail chicken meat, chicken bedding and animal feed (Fig. 3).

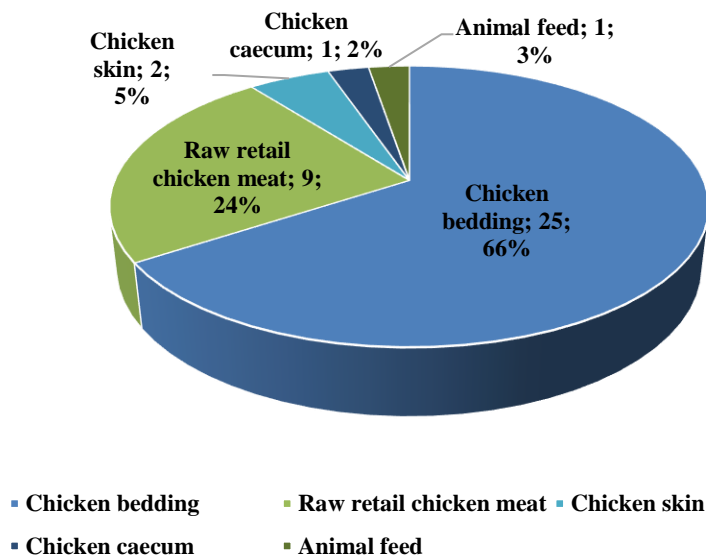


Figure 3. *Salmonella* isolates origin. This graph shows the origin of the 38 *Salmonella* isolates tested with the isolated phages.

The *Salmonella* isolates that could be serotyped belong to 10 serovars, the majority being Infantis (66%) (Fig. 4).

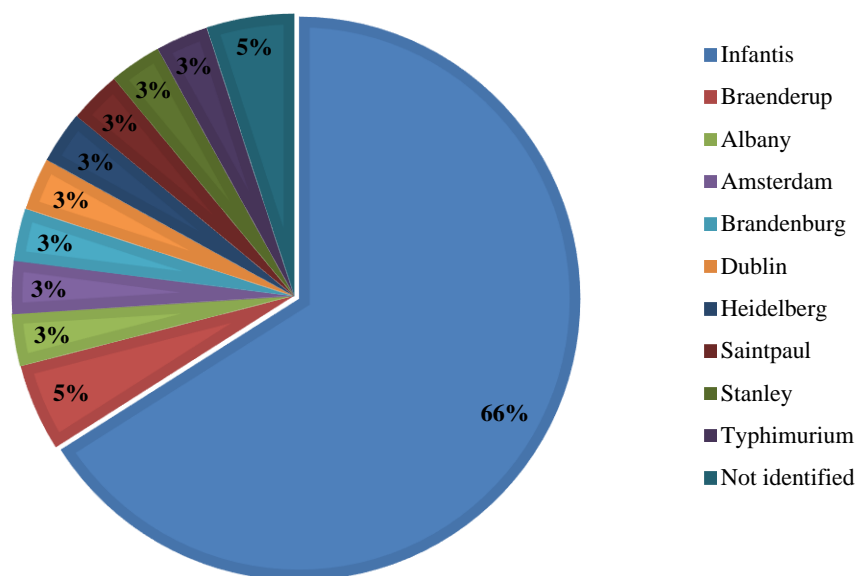


Figure 4. *Salmonella* serovars. This graph shows the serovar distribution of the 38 *Salmonella* isolates tested with phages.

Antimicrobial resistance patterns of Salmonella isolates

Of the 38 *Salmonella* isolates tested, 31 (82%) were MDR. For serovar Infantis, which was the most frequent, 96% of the isolates (24/25) were MDR. The serotypes that showed a pattern of susceptibility to the antimicrobials used were Braenderup, Saintpaul, Amsterdam and Albany. Table 3 shows the antimicrobial resistance patterns of the *Salmonella* isolates, and Fig. 5 also shows their origin.

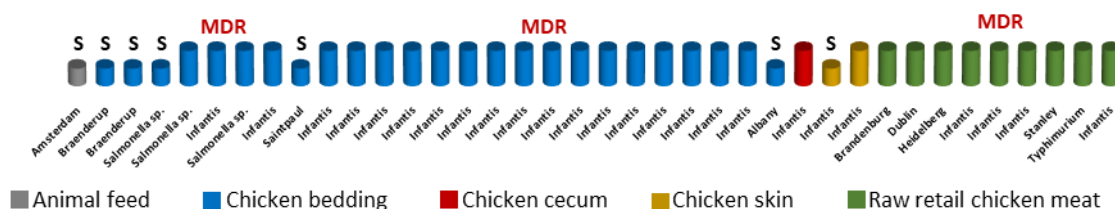
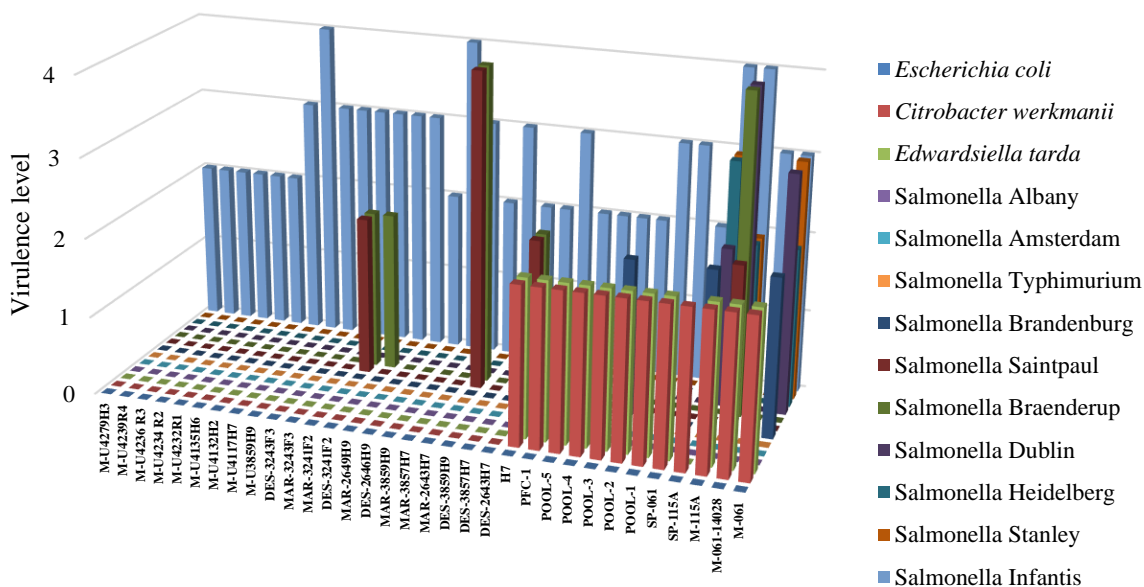


Figure 5. Antimicrobial resistance patterns and origin of *Salmonella* isolates.

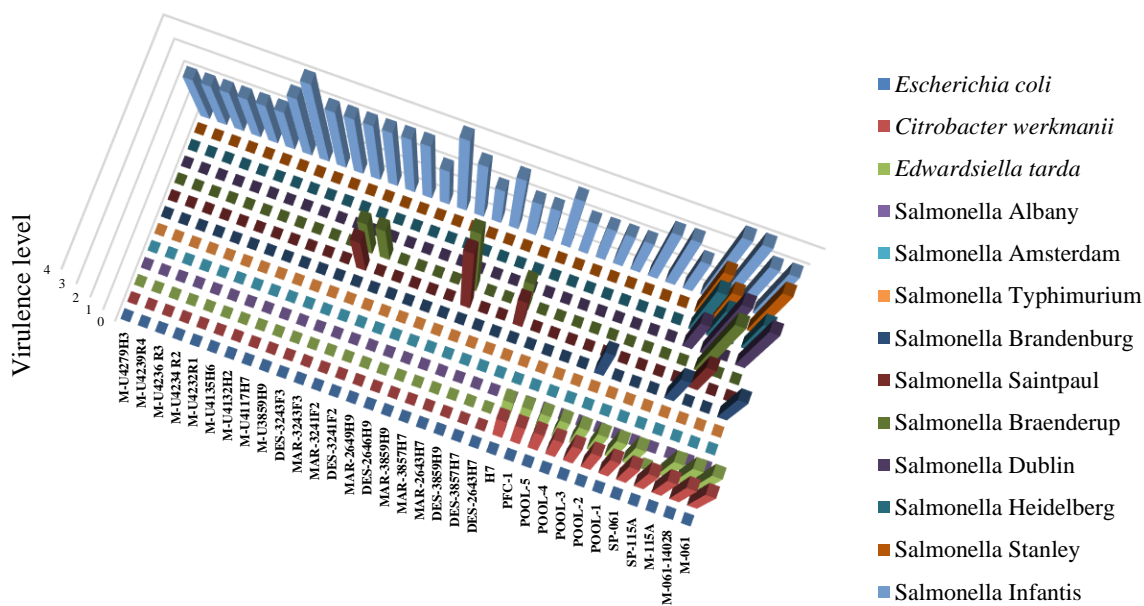
Host range of the isolated phages

The isolated phages showed lytic activity *in vitro* against 84% of the *Salmonella* isolates, and different virulence levels. For on-farm application, the phage mixture M-115A was used, which presented the highest levels of virulence against *Salmonella* isolates (Figs. 6A and 6B).



Phage mixtures

Figure 6A.



Phage mixtures

Figure 6B.

Figure 6. Host range of *Salmonella* phage mixtures. Virulence scale: 4, Complete clearing; 3, total clearing with pinpoint colonies within the plaques; 2, turbidity throughout the cleared zone; 1, a few individual plaques; 0, no clearing (Huang *et al.*, 2018). *Escherichia coli* was isolated from poultry bedding; *Citrobacter werkmanii* and *Edwardsiella tarda*, from a poultry slaughter plant.

Phage-resistant Salmonella isolates

Seven *Salmonella* isolates belonging to serovars Infantis, Albany, Amsterdam, Brandenburg, and Typhimurium were phage-resistant. Table 4 shows the characteristics of these isolates.

Effectiveness of the on-farm application of phages

In the first trial, phages were administered orally with drinking water to chickens from houses that were positive for *Salmonella*. The analysis of the chickens' caecum on day 3 after phage application was negative for *Salmonella*, but on day 9 the presence of the pathogen was detected in the poultry intestine (Fig. 7).

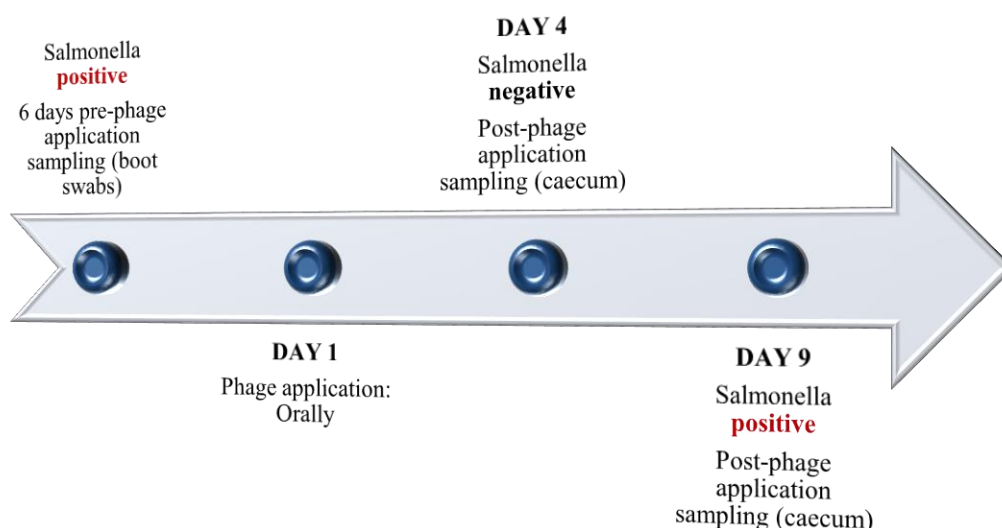


Figure 7. Results of the first trial of on-farm phage application. Phage application effectiveness over time. One application route: Orally (with drinking water).

In the second trial, the phage application was carried out orally with drinking water, and by spraying in the chickens bedding. In this trial, the analysis of the chickens' caecum was negative for *Salmonella* in the samples taken on days 4 and 10 (Fig. 8). The latter corresponded to the slaughter age of the chickens (42-day old).

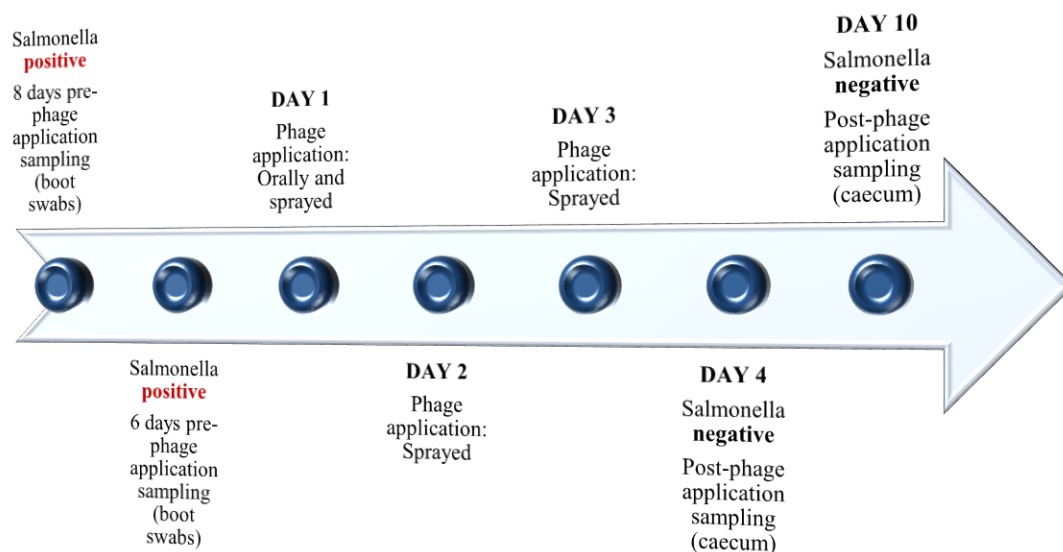


Figure 8. Results of the second trial of on-farm phage application. Phage application effectiveness over time. Two application routes: Orally (with drinking water) and sprayed.

Purification of individual phages from a mixture

The individual phages of the M-061 mixture were purified. Two phages were obtained, named as Salmonella phage M061_16 y Salmonella phage M061_17 (Adriaenssens & Brister, 2017). On the DLA, these phages showed lysis plaques with complete clearance (virulence grade 4) (Huang *et al.*, 2018), and titers of 10^{12} PFU/mL for Salmonella phage M061_16, and 10^8 PFU/mL for Salmonella phage M061_17 were obtained (Fig. 9).

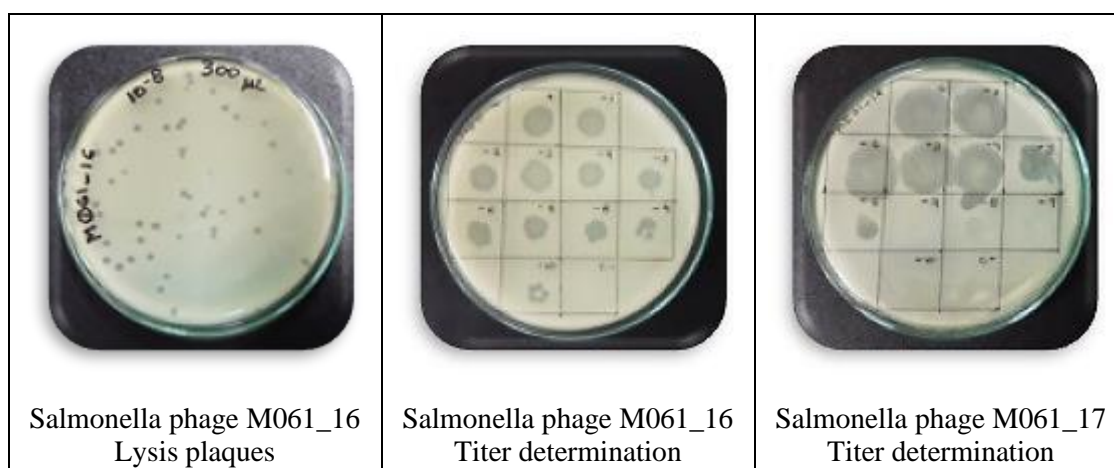


Figure 9. Lytic activity and titer of individual phages. Left: Lysis plaques of Salmonella phage M061_16 suspension. Center and right: Titer spot test of Salmonella phage M061_16 and Salmonella phage M061_17 suspensions.

Transmission electron microscopy

In the micrograph of *Salmonella* phage M061_16 one morphology was observed, while in that of *Salmonella* phage M061_17, more than one morphology, evidencing more than one type of phage in the sample. For the classification of the observed phages, the following parameters were determined: head length (hl; along the axis of the tail), head diameter (hd; width perpendicular to the tail), tail length (tl) and tail diameter (td). Particles with $tl < 40$ nm were classified as *Podoviridae*, and the td value was used to classify viruses with longer tails as *Myoviridae* ($td \geq 16$ nm) or *Siphoviridae* ($td < 16$ nm) (Ackermann, 1999; Jurczak-Kurek *et al.*, 2016). Phages of the *Podoviridae* family, of different sizes, were observed in the *Salmonella* phage M061_16 suspension. Phages of the *Siphoviridae* and *Podoviridae* families were observed in the *Salmonella* phage M061_17 suspension (Table 5).

Discussion

As in other studies, our phages with the best lytic activity were isolated from river water (Machángara) and, because it crosses urbanized and industrial areas, contains wastewater (Higgins *et al.*, 2005; Turki *et al.*, 2012; Huang *et al.*, 2018; Var, Heshmati & AlMatar, 2018). Phages from samples related to poultry operations showed less lytic activity than those from rivers, probably because some of our *Salmonella* isolates are persistent in farms and other poultry processing facilities and could have developed phage-resistance (Wang *et al.*, 2019; Liu *et al.*, 2021), since the majority (65%) of *Salmonella* hosts for phage isolation come from poultry farms.

Knowing the phages host range is important in predicting how microbial communities shape, virulence genes that could be transferred between bacterial species, and the usefulness of phages in controlling pathogenic bacteria. In nature, phages show a great variability of

specificity, from narrow host ranges within a single species to those that can infect more than one bacterial genus (Koskella & Meaden, 2013). All our phages have a narrow, strain-specific host range (Figs. 6A and 6B) because, although they all infect *Salmonella* Infantis, we had 3 *S. Infantis* isolates phage-resistant (Table 4).

Considering the phage application techniques suggested for commercial products and used in other studies (Table 1), we tested two routes of phage application: oral, with drinking water and by spraying on the chicken bedding. The phage mixture M-115A, which showed the highest lytic activity against *Salmonella* was used for on-farm application. The most effective method of phages application was the combination of two routes: oral with drinking water, and by spraying on the chicken bedding, eliminating *Salmonella* in the chickens' caecum until day 10 (Fig. 8). We attribute the success of the methods combination to two factors: 1) The phage concentration increased in the chickens' house (Hagens & Loessner, 2010), and 2) the phages were applied directly on the chicken bedding, where *Salmonella* was detected (Figs. 7 and 8). When only the oral route was used, the detection of the pathogen was negative on day 4, but it was positive again on day 9 (Fig. 7). Apparently, the pathogen remained in the chicken bedding and reinfected the poultry and may also have been spread by coprophagy (Carvalho *et al.*, 2010). But when spraying phages was applied, *Salmonella* was eliminated from the bedding and there was no reinfection of the chickens.

It is inevitable to find bacterial host resistance to phages when using them as antimicrobials (Fong *et al.*, 2020). In our *in vitro* tests, seven *Salmonella* isolates showed resistance to the 34 phage mixtures. These isolates belong to different serovars, and come from chicken bedding (4/7), raw retail chicken meat (2/7), and animal feed (1/7); 5 were MDR, while 2 were susceptible to most of the tested antimicrobials (Table 4). Even though there are reports of cross-resistance to phages and antibiotics (Kortright *et al.*, 2021), generally the bactericidal

mechanisms are different (Loc-Carrillo & Abedon, 2011), and as far as we know it is not common to find this type of resistance.

Although resistance to lytic phages is not common, increases in the population of phage-resistant *Salmonella* have been reported after continuous exposure, so it is recommended that applications be made shortly before poultry slaughtering (Carvalho *et al.*, 2010; Wessels *et al.*, 2021). In accordance with this recommendation, our phages on-farm application started only 10 days before chicken slaughter. However, reports of resistance highlight the need for legislation and monitoring the spread of phage-resistant bacteria (Wessels *et al.*, 2021).

Our results confirm that phages can be used successfully to control *Salmonella* in commercial poultry farms, which constitutes a viable alternative to the use of antibiotics in this field, as there is worldwide concern about the emergence of MDR *Salmonella* serotypes in the food chain (Nair, Venkitanarayanan & Kollanoor, 2018; Xu *et al.*, 2020). This was also evident in our study; among the tested *Salmonella* isolates (whose origin was the poultry industry) a high AMR was found (82%), and among those of the Infantis serotype, 96% was MDR, data that agrees with other reports (García-Soto *et al.*, 2020; Mejia, Vela & Zapata, 2020; Tyson *et al.*, 2020).

On the other hand, in the current poultry production dynamics, newborn chicks are not exposed to the protective commensal microbiota of adult birds, because they are born in the clean environment of an incubator (Litvak *et al.*, 2019); in this context, the use of phages in chickens is proposed at an early age, to prevent colonization of the intestinal tract with *Salmonella* (Bardina *et al.*, 2012).

The purification of individual phages by the DLA technique is based only on the lysis plaques morphology (Piuri, 2019). In our case, the *Salmonella* phage M061_17 suspension could not be purified by this technique, despite having made 5 successive purifications, unlike other studies in which individual phages were obtained with only 3 purifications (Huang *et al.*, 2018;

Hyman, 2019). Electron micrographs allowed us to classify the *Salmonella* phage M061_16 within *Podoviridae* family, and the phages of *Salmonella* phage M061_17 suspension, within *Siphoviridae* and *Podoviridae* families (Table 5). Our results coincide with the described *Salmonella* phages, which correspond to the order *Caudovirales* (Bardina *et al.*, 2012; Moreno Switt *et al.*, 2013; Huang *et al.*, 2018; Var *et al.*, 2018).

One strength of this research was that because it was carried out in collaboration with a commercial farm, the phages were applied at the age when chickens are usually slaughtered, and our data are easily applicable to other poultry farms.

The present investigation also had limitations: (1) We did not have positive controls, that is, sheds in which *Salmonella* was detected, but no phages were applied; and (2) the efficacy of administering the phages to chickens with drinking water and feed, and different concentration of phage in water, was not compared.

Conclusions

The wastewater from the Machángara and San Pedro rivers, in the city of Quito, was a good source of lytic phages against *Salmonella* strains isolated from the local poultry industry. *In vitro*, our phages were lytic against 82% of the tested *Salmonella* isolates, which belong to the *Infantis*, *Saintpaul*, *Braenderup*, *Dublin*, *Heidelberg*, and *Stanley* serovars. All our phages showed lytic activity against *Salmonella* *Infantis*, although we had 3 phage-resistant isolates of *S. Infantis*. This serovar is important in poultry production in Ecuador, due to its high prevalence and AMR. We conclude that the use of phages to control *Salmonella* in poultry production is a viable and safe alternative to the use of antibiotics.

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Tables (Chapter 3)

Table 1. Some examples of *Salmonella* bacteriophages applications in the poultry industry

Antimicrobial	Application substrate	Application technique	Location	Reference
<i>Salmonella</i> phage suspension	Chicken skin	Spray	UK	Goode, Allen & Barrow (2003)
Pool of three <i>Salmonella</i> bacteriophages (isolated from faeces of free-range chickens)	Chicken cuts	Bacteriophages suspended in one liter of buffer were put in contact with chicken parts	Brazil	Fiorentin, Vieira & Barioni Júnior (2005)
<i>Salmonella</i> phage cocktail (3 lytic phages)	10-day old chicks	By coarse spray or drinking water	Valparaíso, Chile	Borie <i>et al.</i> , (2008)
SalmoFREE® Sciphage (6 lytic phages; for therapy and control <i>Salmonella</i>)	Poultry farm	Delivered to the animals via the drinking water	Bogotá, Colombia	Clavijo <i>et al.</i> (2019)
Phage cocktails (4 phages)	Broilers, 20-35 days old	Orally, with drinking water	Santo Domingo de los Tsáchilas, Ecuador	Coba (2020)
<i>Salmonella</i> phage SM1	Poultry litter	Dripping of the phage solution	Brazil	Rogovsky <i>et al.</i> (2021)
Bacteriophage solution (<i>Salmonella</i> specific)	Feathers of live poultry preslaughter	Spray or fine mist application, or wash	NA	Wessels <i>et al.</i> (2021)

NA: Data not available.

Table 2. Origin and number of the isolated *Salmonella* phage mixtures

Phage mixture	Host bacterium origin		
	Source	Raw retail chicken meat	Poultry farms
Machángara River	2 ^I	10 ^I	1
San Pedro River	2 ^I	0	0
Chicken bedding	0	0	2
Chicken slaughter plant water	0	0	4
Chicken house washing water	0	0	1
Chicken cutting machine washing water	0	6 ^{I,B,S}	0
Chicken marinating machine washing water	0	6 ^{I,B,S}	0

Superscripts correspond to the initial of the host *Salmonella* serovar: *I*, Infantis; *B*, Braenderup; *S*, Saintpaul.

Table 3. Antimicrobial resistance patterns of *Salmonella* isolates

Isolate number	<i>Salmonella</i> serovar	SXT	CTX	TE	AZM	CIP	AM	GM	C	NA	CRO	AMC	S
1	Braenderup	S	S	S	S	I	S	S	S	S	S	S	S
2	Braenderup	S	S	S	S	S	S	S	S	S	S	S	S
3	<i>Salmonella</i> sp.	S	S	S	S	S	S	S	S	S	S	S	S
4	<i>Salmonella</i> sp.	S	R	R	S	I	R	S	S	R	R	S	I
5	Infantis	S	R	R	S	I	R	S	S	R	R	S	I
6	<i>Salmonella</i> sp.	S	R	R	S	I	R	S	S	R	R	S	I
7	Infantis	S	R	R	S	I	R	S	R	R	R	S	S
8	Infantis	S	R	R	S	S	R	S	S	R	R	R	I
9	Saintpaul	S	S	S	S	S	S	S	S	S	S	S	S
10	Infantis	S	R	R	S	I	R	S	S	R	R	S	R
11	Infantis	R	R	R	S	I	R	I	S	R	R	S	I
12	Infantis	R	R	R	S	I	R	I	S	R	R	S	R
13	Infantis	R	R	R	S	I	R	I	R	R	R	S	I
14	Infantis	S	R	R	S	I	R	S	S	R	R	S	I
15	Infantis	S	R	R	S	I	R	S	S	R	R	S	R
16	Infantis	S	R	R	S	I	R	S	S	R	R	S	R
17	Infantis	S	R	R	S	I	R	S	S	R	R	S	I
18	Infantis	R	R	R	S	I	R	I	S	R	R	S	R
19	Infantis	R	R	R	S	I	R	I	R	R	R	S	R
20	Infantis	R	R	R	S	I	R	I	R	R	R	S	I
21	Infantis	S	R	R	S	I	R	S	S	R	R	S	R
22	Amsterdam	S	S	S	S	I	S	S	S	S	S	S	S
23	Infantis	S	S	S	S	S	S	S	S	S	S	S	S
24	Infantis	R	R	R	S	I	R	I	R	R	R	S	R
25	Infantis	S	R	R	S	I	R	I	R	R	R	S	R
26	Infantis	S	R	R	S	I	R	S	S	R	R	S	R
27	Infantis	S	R	R	S	S	R	S	S	R	R	R	R
28	Infantis	S	R	R	R	I	R	S	I	R	R	S	R
29	Albany	S	S	S	S	S	S	S	S	S	S	S	I
30	Brandenburg	R	R	R	S	I	R	I	R	R	R	S	R
31	Dublin	R	R	R	S	I	R	S	S	R	R	S	R
32	Heidelberg	R	R	R	S	I	R	I	R	R	R	S	R
33	Infantis	S	R	R	S	I	R	S	S	R	R	S	na
34	Infantis	R	R	R	S	I	R	I	R	R	R	S	na

35	Infantis	R	R	R	S	I	R	I	R	R	R	S	na
36	Stanley	R	R	R	R	I	R	I	R	R	R	S	I
37	Typhimurium	S	S	S	R	I	R	S	S	S	S	R	na
38	Infantis	R	R	R	S	I	R	I	R	R	R	S	na

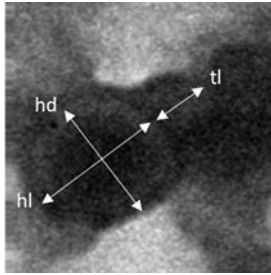
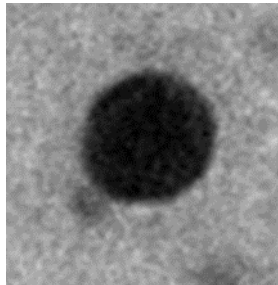
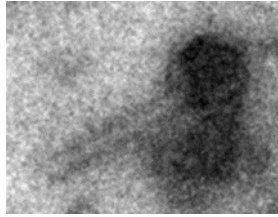
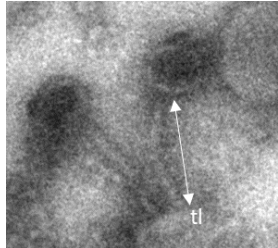
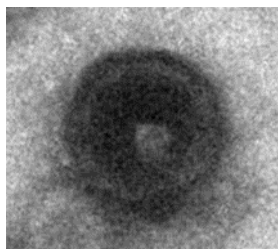
Abbreviations: I, intermediate; R, resistant; S, susceptible; na, data not available; AM, ampicillin; AMC, amoxicillin-clavulanic acid or amoxicillin-clavulanate; AZM, azithromycin; C, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CRO, ceftriaxone; GM, gentamicin; NA, nalidixic acid; TE, tetracycline; S, streptomycin; SXT, sulphamethoxazole-trimethoprim (CLSI, 2021). The trial was performed once ($n = 1$).

Table 4. Characteristics of the phage-resistant *Salmonella* isolates.

<i>Salmonella</i> isolate number	Serovar	Origin	Antimicrobial resistance pattern
8	Infantis	Chicken bedding	CTX, TE, AM, NA, CRO, AMC, S(I)
26	Infantis	Chicken bedding	CTX, TE, AM, NA CRO, S
27	Infantis	Chicken bedding	CTX, TE, AM, NA, CRO, AMC, S
22	Amsterdam	Animal feed	CIP(I)
29	Albany	Chicken bedding	S(I)
31	Brandenburg	Raw retail chicken meat	SXT, CTX, TE, AM, C, NA, CRO, S, CIP(I), GM(I)
38	Typhimurium	Raw retail chicken meat	AZM, AM, AMC, CIP(I)

Abbreviations: I, intermediate resistance; AM, ampicillin; AMC, amoxicillin-clavulanic acid or amoxicillin-clavulanate; AZM, azithromycin; C, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CRO, ceftriaxone; GM, gentamicin; NA, nalidixic acid; TE, tetracycline; S, streptomycin; SXT, sulphamethoxazole-trimethoprim (CLSI, 2021).

Table 5. Classification of the phages observed in photomicrographs.

Phage name	Phage family (hl, hd, tl, td) (nm)	Electron photomicrograph
Salmonella phage M061_16_1	<i>Podoviridae</i> (102, 98, 39, 45)	
Salmonella phage M061_16_2	<i>Podoviridae</i> (82, 72, 20, 18)	
Salmonella phage M061_17_1	<i>Siphoviridae</i> (66, 65, 148, 9)	
Salmonella phage M061_17_2	<i>Siphoviridae</i> (55, 68, 140, 13)	
Salmonella phage M061_17_3	<i>Podoviridae</i> (118, 119, 22, 33)	

Supplementary Material (Chapter 3)

Table S1. Data from phage source samples

Sample type	Origin (location)	Sample number/quantity	Sampling date
Wastewater	Machángara river (Quito, Ecuador)	1 sample (ca 1 US gal)	February 2019
Wastewater	San Pedro river (Quito, Ecuador)	1 sample (ca 1 US gal)	February 2019
Chicken bedding samples	Poultry farms (Pichincha Province, Ecuador)	6 samples from 6 chicken houses (two composite samples were made, each one with samples from 3 houses)	May 2019
Washing water	Poultry slaughter plant (Pichincha Province, Ecuador)	8 samples: Pre-chiller (1, chicken), chiller (2, chicken and turkey), scalding (2, chicken and turkey), gutting machines (2, chicken), and final poultry washing (1, turkey)	June 2019
Washing water	Poultry processing plant (Quito, Ecuador)	2 samples: Poultry cutting and marinating machines	August 2019

CHAPTER 4

General discussion

From the results of the systematic review on nontyphoidal *Salmonella* (NTS) in foods from Latin America, there are two food groups in which the presence of this pathogen is particularly alarming, and it is urgent to combat it: Meat products, and fresh fruits and vegetables.

Meat products, for two reasons: (1) The highest prevalence of NTS (> 70%) were found in these foods, and (2) the high incidence of multidrug-resistant (MDR) isolates, since 90% of the reports of antimicrobial resistance (AMR) were related to meats and derived products. NTS contaminates meat because healthy animals can carry this pathogen and their carcasses become contaminated with gastrointestinal content during slaughter (Gómez-Aldapa *et al.*, 2012; Contreras-Soto *et al.*, 2019), while the high AMR rate reflects the selective pressure exerted on bacteria by the misuse of antimicrobials in animal husbandry (Silva, Calva & Maloy, 2014; McDermott, Zhao & Tate, 2018). AMR in animal foods should constitute an urgent call for attention to an increasing worldwide problem: the spread of MDR isolates through food and its contribution to the global problem of AMR (Mejía, Vela & Zapata, 2020; Vidovic & Vidovic, 2020; CDC, 2021). To achieve a reduction of antimicrobial use in food production, the participation of governmental and non-governmental organizations in charge for the control and training of producers is required. It is also necessary the commitment of the producers of foods of animal origin (including farmers, poultry farmers, fish farmers, etc.) to reduce antimicrobial use in their activities. Finally, the involvement of academia would be desirable, sharing knowledge with the productive sectors, and contributing with accessible solutions (de Freitas *et al.*, 2010; Vidovic & Vidovic, 2020).

Fresh fruits and vegetables are increasingly implicated in salmonellosis outbreaks and are considered an important route of entry of NTS in the food chain. Furthermore, MDR bacterial strains have also been reported in these foods (Cordano and Virgilio, 1996; Almeida *et al.*, 2018; Montero *et al.*, 2021).

If *Salmonella* was originally adapted to the microenvironment of the animals' gastrointestinal tract, what are the pathways and mechanisms for this bacterium to be so successful in vegetables? There are numerous studies that contribute on answering this question.

Once *Salmonella* leaves the animal gastrointestinal tract, it can survive and even multiply in faecal matter, allowing it to increase its population and the chances of reaching other hosts (Guerrero *et al.*, 2020). In low- and middle-income countries, manure is used as fertilizer without adequate treatment, constituting a potential source of *Salmonella* for produce (Gallegos-Robles *et al.*, 2008; Fletcher *et al.*, 2013). Through its dispersion in the environment, this pathogen can also reach surface waters that, in addition to protecting it from solar UV radiation and changes in pH and temperature, are often used for irrigation and are the vehicle for this pathogen to reach crops (Liu, Whitehouse & Li, 2018).

The surface of some rough fruits and vegetables provides a niche for *Salmonella* and if their population is large enough, consumption of the agricultural product can cause human disease. On the other hand, this pathogen can also enter the leaves through structures such as trichomes and stomata, and benefit from nutrients resulting from the degradation of plant tissues by phytopathogenic bacteria that thus promote the growth of *Salmonella* in the plant and its colonization, and the vegetable becomes an alternative host (Liu *et al.*, 2018). Hence the importance of preventing the pathogen from reaching crops, because if it penetrates plant tissues, subsequent disinfection treatments could be ineffective (da Cruz *et al.*, 2019).

To effectively prevent contamination of crops with *Salmonella* it is important to know its physiology outside of an animal host. For this purpose, we carried out a series of experiments

to determine if this pathogen can replicate in faecal matter outside a host. Our results showed that *Salmonella* multiplies massively and through aerobic respiration in chicken fresh faecal matter, which agrees with the findings of Litvak and colleagues (2019), who found that *Salmonella* Enteritidis requires an increase in epithelial oxygenation in the chicken's intestine for expansion by aerobic respiration (Litvak *et al.*, 2019). We also found that the growth of NTS increases when that of *E. coli* decreases, suggesting a possible antagonism between these two bacteria, which could be due to competition for oxygen. Similarly, the results of Litvak (2019) suggest that *S. Enteritidis* competes with commensal *Enterobacteriaceae* for oxygen (Litvak *et al.*, 2019).

Our results showed critical implications in procedures related to food production: (1) It is essential to treat manure to eliminate *Salmonella* and other pathogens before using it as fertilizer; composting (Singh, Kim, & Jiang, 2012; Román, Martínez, & Pantoja, 2015) or procedures that generate anaerobic conditions, as anaerobic lagoons and digesters (Spiehs & Goyal, 2007) can be used for this purpose. (2) When there are faeces residues, *Salmonella* can multiply in chicken bedding and spread among poultry. The bedding material is often used raw or after improper composting, as fertilizer (Chen & Jiang, 2014), producing food crops contamination. Bodi and colleagues (2013) found that the aeration by tumbling of the chicken litter can increase the multiplication and dissemination of *Salmonella* when there is high poultry population density, improper ventilation of the facilities, or the presence of humidity and excrement of *Salmonella* (Bodi *et al.*, 2013). These findings are consistent with our conclusions that the presence of oxygen favors the multiplication of *Salmonella* in the presence of faecal matter (Guerrero *et al.*, 2020). For this reason, it is necessary to look for cleaning and disinfection procedures that do not create favorable conditions for its growth.

NTS is the leading bacterial cause of foodborne illness worldwide (CDC, 2020). Although most cases of salmonellosis in humans are mild, foodborne illness causes economic losses for

governments, producers, and consumers (FAO, 2020). To control this pathogen in food animals' production, bacteriophages (phages) are a viable option, considering the current restrictions on the use of antimicrobials in animal husbandry. Phage safety, compared to antimicrobials, is due to its specificity of host bacteria, since in general, each type of phage has the capacity to infect only one species, serotype or strain, and does not affect the commensal microbiota (Hagens, & Loessner, 2010; Wernicki, Nowaczek, & Urban-Chmiel, 2017).

There are pros and cons of using phages as antibacterials in the food industry (Hagens, & Loessner, 2010; Loc-Carrillo, & Abedon, 2011), as presented below (Fig. 1).

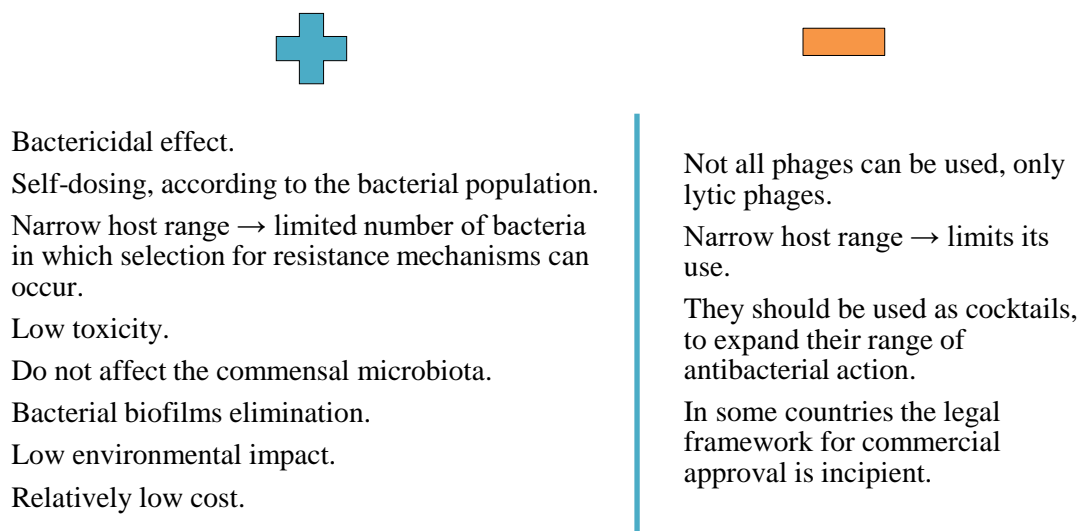


Figure 1. Summary of pros and cons of phages as antimicrobials in the food industry previously stated by Hagens & Loessner (2010), and Loc-Carrillo & Abedon (2011).

In several countries phages are being used in the food animals' husbandry and applied on the surface of foods of animal and vegetable origin, to eliminate pathogens (Wernicki *et al.*, 2017). Despite their safety, it is recommended that the phages for use food have the following properties: (1) "Wide" host range, that is, they infect some members of the species or genus to be eliminated; (2) be lytic (virulent); (3) can be propagated in a non-pathogenic host; (4) known complete genome to ensure absence of pathogenicity genes; (5) stable during storage and application; (6) not cause adverse effects when administered orally; and (7) GRAS approval for

use in food (Hagens & Loessner, 2010). In the present work, some of the requirements were verified to apply *Salmonella* phages on-farm, others are in process. The verified requirements are: 1) The use of lytic phages for on-farm application; 2) phages were propagated in *Salmonella* isolates from raw chicken meat, but prior to application, phage suspensions were filtered to remove bacterial contamination; 3) it was confirmed that phages were not toxic to chickens. The genomic characterization of our phages is in progress, and we need to do stability tests during their storage and application.

A widespread application of phages in food production could also have negative consequences if control measures are not taken. These consequences could be the uncontrolled spread of phages, and the persistence and dispersal of bacterial isolates resistant to them (Sommer *et al.*, 2019); it has been seen that with prolonged treatments the appearance of isolates resistant to phages is more likely (Carvalho *et al.*, 2010). In our on-farm applications, the longest period between oral phage administration and chickens slaughter was 10 days; thus, our intervention would not be a risk factor for the proliferation of phage-resistant isolates. Regarding the application in chicken bedding, it is necessary to develop a cleaning and disinfection plan that eliminates *Salmonella* and, consequently, phages, and periodically verify its compliance and effectiveness.

We had longer poultry protection against *Salmonella* by combining oral phage administration and spraying, however, we did not compare the effectiveness of these two routes separately. Borie and colleagues (2008) compared these two application routes and obtained better results using spraying (phages isolated from sewage samples) (Borie *et al.*, 2008). On the other hand, unlike Clavijo and colleagues (2019), who eliminated 100% of *Salmonella* in poultry using a commercial phage cocktail orally in multiple doses (a weekly dose for three weeks) (Clavijo *et al.*, 2019), we controlled *Salmonella* with a single application combining via oral and spraying.

In summary, proper waste treatment is critical to avoid contamination of crops with pathogens such as NTS. In food animals' husbandry, phage application is a viable option for the control of this pathogen, instead of antimicrobials. It is important to reduce the on-farm incidence of NTS because it diminishes its detection in slaughterhouses and, consequently, in food, which protects consumers from disease.

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FINAL REMARKS

Conclusions

The importance of nontyphoidal *Salmonella* (NTS) in food production and the difficulty of its control in the poultry industry is well known; the main factor for NTS entering the food chain is its presence on farms (Pelyuntha *et al.*, 2021). In Latin America, animal foods showed the highest prevalence of NTS, and multidrug-resistant (MDR) isolates have been reported with more frequency in this food. In Ecuador, NTS is present in 88% of chicken carcasses, and 91% of strains are MDR (Vinueza-Burgos *et al.*, 2019).

Once excreted, *Salmonella* multiplies massively in chicken faecal matter, which constitutes its intermediate habitat (Barrera *et al.*, 2018). This behavior appears to increase the chances for NTS to reach new hosts. Based on this knowledge, effective measures can be taken to prevent this pathogen from contaminating food sources.

With the alarming increase in bacterial resistant to antimicrobials and the consequent regulation in their use, bacteriophages (phages) constitute an alternative for pathogens control. We were successful in eliminating *Salmonella* by applying on-farm lytic phages isolated from the Machángara river (Quito). The best method was the combination of oral administration to chickens with drinking water and spraying on chicken bedding for three days. Our conclusion is that phages are a viable alternative, due to their safety and relatively low cost, to control *Salmonella* in poultry production. The emergence of phage-resistant *Salmonella* strains should be constantly monitored to seek new phages that eliminate them, and to implement strict disinfection protocols in all facilities. In addition, as its use spreads, processes should be implemented that allow the production of phages to be scaled up to levels higher than in the laboratory, which was how this research was carried out.

Recommendations

Systematic review

It is recommendable to limit the bibliographic search to a topic as specific as possible. The narrower the range of knowledge covered, the deeper the information can be and the more applicable the results.

Experiments on the multiplication of Salmonella in chicken faecal matter

- (1) It would be interesting to test whether NTS has the same behavior in the faecal matter of other animals that are reservoirs of different *Salmonella* serovars.
- (2) A complement to our work would be to determine the metabolic pathways used by the pathogen in these experiments, using mutant strains for the pathways under study (Litvak *et al.*, 2019).

Bacteriophages for the control of Salmonella in poultry

- (1) To compare the efficacy of administering phages with drinking water and with food. Food has been found to protect phages from being inactivated by low pH throughout the poultry digestive tract (Carvalho *et al.*, 2010).
- (2) In addition to performing the qualitative *Salmonella* detection to evaluate the phage efficacy, quantify their population before and after the phage application, to compare different treatments and concentrations (Xie *et al.*, 2021).
- (3) To confirm that phages cannot perform a lysogenic cycle, to ensure that they are exclusively lytic (Hagens & Loessner, 2010).
- (4) To combine two or more antibacterial agents against *Salmonella* (phages, probiotics, feed acidification) in the administration to chickens, to determine if there is a synergistic effect (Clavijo & Vives, 2017).

Future perspectives

The present research has future perspectives, as it is the beginning of a project that continues to be developed, based on the application of *Salmonella* phages on-farm. Through electron microscopy, the characterization of the individual phages began but there are further steps to pursue; the next step will be genomic characterization. Additionally, phages that kill resistant *Salmonella* strains (Table 6, Chapter 3) will be isolated from new environmental samples.

In the short term, it would be desirable to define all conditions of phage on-farm application in various poultry farms (related to the farm where the application was carried out).

In the medium and long term, the expectation is to totally replace the use of antibiotics in the poultry industry in Ecuador, and phages could be part of the strategy to achieve this ambitious goal.

Limitations

The main limitation was not having experimental units (groups of chickens that receive different treatment) to test the phage effectiveness, so the respective controls were not available and the necessary repetitions for a statistical analysis of the data could not be carried out.

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