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ORIGINAL ARTICLE

Genomic characterization and antimicrobial resistance profiles of *Salmonella enterica* serovar Infantis isolated from food, humans and veterinary-related sources in Brazil

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Abstract

Aims: To characterize the genetic relatedness, phenotypic and genotypic antimicrobial resistance and plasmid content of 80 *Salmonella Infantis* strains isolated from food, humans and veterinary sources from 2013 to 2018 in Brazil.

Methods and results: Pulsed-field gel electrophoresis and single-nucleotide polymorphism analysis showed major clusters containing 50% and 38.8% of the strains studied respectively. Multilocus sequence typing assigned all strains to ST32. Disk-diffusion revealed that 90% of the strains presented resistant or intermediate resistant profiles and 38.8% displayed multidrug resistance. Resistance genes for aminoglycosides (*aac(6′)-Iaa*; *aadA12*; *aph(3″)-Ib*; *aph(6)-Id*), β-lactams ($bla_{\text{TEM-1}}$; $bla_{\text{CTX-M-8}}$; $bla_{\text{CMY-2}}$), trimethoprim (*dfrA8*), tetracycline (*tet(A)*), amphenicols (*floR*), sulfonamide (*sul2*), efflux pumps (*mdsA*; *mdsB*), chromosomal point mutations in *gyrB*, *parC*, *acrB* and *pmrA* were detected. Strains harboured IncI, IncF, IncX, IncQ, IncN and IncR plasmids.

Conclusions: The presence of a prevalent *S*. Infantis subtype in Brazil and the high antimicrobial resistance rates reinforced the potential hazard of this serovar for the public health and food safety fields.

Significance and Impact of the Study: This is the first study characterizing a large set of *S*. Infantis from Brazil by whole-genome sequencing, which provided a better local and global comprehension about the distribution and characteristics of this serovar of importance in the food, human and veterinary fields.

KEYWORDS

antimicrobial resistance genes, multilocus sequence typing, plasmids, pulsed-field gel electrophoresis, *Salmonella* Infantis, whole-genome sequencing

INTRODUCTION

Infections caused by non-typhoid *Salmonella enterica* serovars are considered one of the four major causes of human foodborne diseases worldwide, accounting for 93.8 million cases of gastroenteritis and 155 thousand deaths each year (Majowicz et al., 2010; WHO, 2020). *Salmonella enterica* subspecies *enterica* serovar Infantis (*S*. Infantis) is a non-typhoid and ubiquitous serovar with global distribution over different isolation sources. Similar to other serovars, the main reservoir of *S*. Infantis are food-producing animals, with high prevalence in poultry, and with swine and bovine sources also playing an important role on its transmission (Carfora et al., 2018; Elbediwi et al., 2021; Kalaba et al., 2017; Shahada et al., 2010; Xu et al., 2021). In humans, the main clinical manifestation due to *S*. Infantis contamination is gastroenteritis, developed by the consumption of contaminated raw or undercooked meat products (Brown et al., 2018; Fonseca et al., 2006; Pessoa-Silva et al., 2002; Ranjbar et al., 2018).

In Brazil, previous reports have demonstrated the high prevalence of this serovar among food, environmental, animal and human sources (Castro et al., 2002; Cunha-Neto et al., 2018; Fonseca et al., 2006; Moraes et al., 2000), which may pose as a major food safety and public health concern due to the country's position as one of the largest meat exporters in the world.

In addition to its broad distribution over many sources and locations, studies also demonstrated increasing antimicrobial resistance rates among *S*. Infantis strains, which may indicate a possible route for the dissemination, transmission and establishment of drug-resistant infections in humans (Acar et al., 2019; Brown et al., 2018; Carfora et al., 2018; Cunha-Neto et al., 2018; Elbediwi et al., 2021; Kalaba et al., 2017; Xu et al., 2021). Although the monitoring of resistance to antimicrobial drugs of choice for the treatment of *Salmonella* infections in humans must be of priority, such as fluoroquinolones and third- and fourth-generation cephalosporins, it is also important to notice the resistance rates to drugs less used in human therapy and employed in the veterinary field for animal therapy or even illegally used in Brazil as growth promoters in food-producing animals, such as aminoglycosides, tetracycline, sulfonamides and amphenicols (Acar et al., 2019; Brown et al., 2018; Carfora et al., 2018; Elbediwi et al., 2021; Kalaba et al., 2017; Ranjbar et al., 2018; Xu et al., 2021). Mobile genetic elements, especially plasmids, are well-known to play a key role in the easy acquisition and dissemination of such antimicrobial resistance genes, as well as genes associated to virulence, survival and fitness among bacteria (Alba et al., 2020; Carattoli et al., 2014; Kürekci et al., 2021; McDermott et al., 2018).

Epidemiological studies based on molecular typing methods have been demonstrated to play an important role in the characterization of *Salmonella* serovars, as well as in the tracking and investigation of related outbreaks (Allard, 2016; Gilmour et al., 2013). In addition, the advances obtained in whole-genome sequencing (WGS) techniques in recent years, associated to a broader access worldwide due to cost reductions, provided major advances in the epidemiological studies of foodborne pathogens through the development of new methods for the monitoring of the genetic correlation, antimicrobial resistance and dissemination of epidemic plasmids among bacterial pathogens (Allard, 2016; Gilmour et al., 2013).

Over the years, methods such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) have been extensively employed with success in studying *Salmonella* serovars, including *S*. Infantis (Almeida et al., 2013; Kürekci et al., 2021; Mejía et al., 2020; Monte et al., 2019; Ranjbar et al., 2018; Xu et al., 2021). Furthermore, genomic analysis based on WGS data, such as the analysis of single-nucleotide polymorphisms (SNPs), have also been used to characterize strains of this serovar (Acar et al., 2019; Alba et al., 2020; Brown et al., 2018).

Despite Brazil's position as a major meat exporter country and the importance that *Salmonella* monitoring should have, few studies have been conducted aiming to characterize specific traits of an expressive number of *S*. Infantis strains circulating in this country. Most of the published reports were usually limited to the isolation and determination of antimicrobial resistance profiles of *S*. Infantis isolates, and a smaller number employed molecular typing methods for its characterization (Almeida et al., 2013; Castro et al., 2002; Fonseca et al., 2006; Moraes et al., 2000; Pessoa-Silva et al., 2002).

In light of this, considering the few information available regarding this serovar of great importance in the food, veterinary, environmental and clinical fields in Brazil and in many countries, the aims of this study were to characterize the genomic relatedness and evaluate the antimicrobial resistance profiles and plasmid frequencies of *S*. Infantis strains isolated from food, farm and industry environments, humans, animals and animal feed from 2013 to 2018 in Brazil.

MATERIAL AND METHODS

Bacterial strains

A total of 80 *S*. Infantis strains from food ($n = 27$), humans ($n = 19$) and veterinary related sources, such as

farm and industry environments ($n = 24$), animals ($n = 7$) and animal feed ($n = 3$) were included in this study. These strains were isolated between 2013 and 2018 from states of the south (Santa Catarina, Rio Grande do Sul and Paraná), southwest (São Paulo and Minas Gerais), midwest (Mato Grosso do Sul and Goiás) and northwest (Alagoas and Maranhão) regions of Brazil. All strains were provided by the *Salmonella* reference laboratory collection of the Oswaldo Cruz Foundation of Rio de Janeiro (FIOCRUZ-RJ). Detailed information regarding the year, source, material and place of isolation of the 80 *S*. Infantis strains studied is displayed in Table 1.

Pulsed-field gel electrophoresis

Agarose plugs containing the genomic DNA of all strains studied were prepared according to the PulseNet protocol as previously described (Ribot et al., 2006) and digested with 40 U of the restriction enzyme *XbaI* (Thermo Fischer Scientific). The standard molecular weight ladder Lambda Ladder PFG Marker (New England BioLabs) was included to allow the comparison of the fingerprints over several gels. Gels were stained with ethidium bromide (1.0 μg/ml) for 30 min and distained in distilled water for 90 min. Restriction fragments were visualized and documented under UV light. The analysis of the PFGE profiles similarity was performed using Bionumerics 7.6 (Applied Maths). A similarity dendrogram was constructed by the UPGMA method using the DICE similarity coefficient and a position tolerance of 1.5%. PFGE's discriminatory power was assessed by Simpson's diversity index (Hunter & Gaston, 1988). PFGE groups were determined in the dendrogram using an 80% similarity cut-off.

Antimicrobial susceptibility test

The phenotypic antimicrobial resistance was determined by the disk-diffusion method for the antimicrobials amoxicillin-clavulanic acid (30 μg), piperacillin (10 μg), ampicillin (10 μg), cefazolin (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg), amikacin (30 μg), gentamycin (30 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), tetracycline (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and chloramphenicol (30 μg). The performing of the disk-diffusion method, selection of antimicrobial agents and interpretation of results were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2019).

Whole-genome sequencing

Genomic DNA isolation was performed by the phenol-chloroform-isoamyl alcohol as previously described (Vilela et al., 2021). Libraries were prepared with 1ng of genomic DNA with the Nextera XT DNA kit (Illumina). Genomes were sequenced in an Illumina MiSeq sequencer using the 2 × 150-bp paired-end MiSeq Reagent Kit version 3 (Illumina). Draft genomes were assembled with SKESA 2.2 and NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) and quality control was performed in MicroRunQC workflow. All information regarding the sequencing of the 80 *S*. Infantis strains, as well as its respective accession numbers, have been previously reported in detail by Vilela et al. (2021).

MLST and SNP analyses

MLST was conducted for the 80 *S*. Infantis strains studied based on the scheme of seven housekeeping genes (*aroC*, *dnaN*, *hemC*, *hisD*, *purE*, *sucA* and *thrA*) for *S. enterica* using the online tool MLST 2.0, available at https://cge.cbs.dtu.dk/services/MLST/ (Larsen et al., 2012).

Two different SNP based approaches were used to evaluate the genomic relatedness of the 80 *S*. Infantis strains analysed in this study (Table 1).

To provide an overview exclusively of the 80 *S*. Infantis strains studied, a SNP analysis using the online tool CSI Phylogeny 1.4 (Call SNPs & Infer Phylogeny) available at https://cge.cbs.dtu.dk/services/CSIPhylogeny/ was conducted using the following default parameters: minimum depth at SNP positions 10×, minimum relative depth at SNP positions 10%, minimum distance between SNPs (prune) 10 bp, minimum SNP quality 30, minimum read mapping quality 25 and minimum *Z*-score 1.96 (Kaas et al., 2014). The phylogenetic tree obtained was visualized and edited using software FigTree v. 1.4.2 (Rambaut Research Group, Institute of Evolutionary Biology, University of Edinburgh). The complete genome of *S*. Infantis reference strain SINFA (Genbank accession number LN649235.1), which was isolated from chicken in the United Kingdom in 1973, was used for the alignment and included for comparison purposes in the phylogenetic tree.

In addition, to verify the genomic relatedness of the 80 *S*. Infantis strains studied with isolates from other countries, its accession numbers (Table 1) were searched using the Isolate Browser of NCBI's Pathogen Detection platform (https://www.ncbi.nlm.nih.gov/pathogens/isolates) to verify the SNP clusters of the strains studied.

TABLE 1 Isolation data and accession numbers of the 80 *Salmonella* Infantis strains studied isolated from food ($n = 27$), the environment ($n = 24$), humans ($n = 19$), animals ($n = 7$) and animal ration ($n = 3$) between 2013 and 2018 in Brazil

	Isolation data			Accession no.	
Strain no.	**State**	**Material**	**Source**	**CFSAN Strain no.**	**GenBank accession no.**
SI 1348/13	PR	Human faeces	Human	CFSAN107127	AAWRHH000000000.1
SI 2385/13	PR	Soy	Food	CFSAN107129	AAWRGU000000000.1
SI 2950/13	AL	Human faeces	Human	CFSAN107130	AAWRHS000000000.1
SI 2951/13	AL	Human faeces	Human	CFSAN107131	AAWRHN000000000.1
SI 3156/13	SC	Disposable shoe cover	Environment	CFSAN107132	AAWRGH000000000.1
SI 5025/13	SC	Human faeces	Human	CFSAN107133	AAWRGA000000000.1
SI 124/14	RS	Swine faeces	Animal	CFSAN107134	AAWRDW000000000.1
SI 210/14	SC	Dragging swab	Environment	CFSAN107136	AAWREM000000000.1
SI 212/14	SC	Dragging swab	Environment	CFSAN107137	AAWRDZ000000000.1
SI 388/14	SP	Soybean animal meal	Animal ration	CFSAN107138	AAWRER000000000.1
SI 583/14	SC	Chicken carcass	Food	CFSAN107139	AAWREP000000000.1
SI 584/14	SC	Pasta containing ham	Food	CFSAN107140	AAWREX000000000.1
SI 677/14	SC	Carcass cleaning wipe	Food	CFSAN107141	AAWRFG000000000.1
SI 723/14	SC	Dragging swab	Environment	CFSAN107142	AAWRFD000000000.1
SI 982/14	RS	Chicken faeces	Animal	CFSAN107143	AAWRHV000000000.1
SI 1143/14	RS	Chicken faeces	Animal	CFSAN107144	AAWRHU000000000.1
SI 1284/14	SC	Dragging swab	Environment	CFSAN107145	AAWRIM000000000.1
SI 1380/14	RS	Chicken faeces	Animal	CFSAN107146	AAWRIF000000000.1
SI 1408/14	RS	Human faeces	Human	CFSAN107148	AAWRIL000000000.1
SI 1409/14	RS	Human faeces	Human	CFSAN107149	AAWRHF000000000.1
SI 1441/14	RS	Mayonnaise	Food	CFSAN107150	AAWRHL000000000.1
SI 1711/14	RS	Chicken faeces	Animal	CFSAN107151	AAYKFJ000000000.1
SI 2378/14	SC	Truck swab	Environment	CFSAN107152	AAWRHR000000000.1
SI 2430/14	SC	Mixed meat sausage	Food	CFSAN107153	AAWRHO000000000.1
SI 2461/14	SC	Chicken carcass	Food	CFSAN107154	AAWRGI000000000.1
SI 2463/14	SC	Chicken carcass	Food	CFSAN107155	AAYKFK000000000.1
SI 2548/14	RS	Chicken faeces	Animal	CFSAN107156	AAWRDS000000000.1
SI 3836/14	RS	Dragging swab	Environment	CFSAN107160	AAXBHC000000000.1
SI 4882/14	MG	Chicken carcass	Food	CFSAN107164	AAXBHW000000000.1
SI 4892/14	MG	Chicken wings	Food	CFSAN107165	AAXAKM000000000.1
SI 4895/14	MG	Chicken carcass	Food	CFSAN107166	AAXAKH000000000.1
SI 4901/14	MG	Pig snout	Food	CFSAN107167	AAXAKN000000000.1
SI 5247/14	MG	Chicken upper leg and thigh	Food	CFSAN107168	AAXAKJ000000000.1
SI 342/15	SC	Swine heart	Food	CFSAN107171	AAXHSY000000000.1
SI 444/15	SC	Pork filet	Food	CFSAN107172	AAXHRH000000000.1
SI 447/15	SC	Smoked and salted pork meat	Food	CFSAN107173	AAXHRI000000000.1
SI 1809/15	SC	Meat animal meal	Animal ration	CFSAN107179	AAXHSE000000000.1
SI 1816/15	SC	Poultry viscera animal meal	Animal ration	CFSAN107180	AAXHVG000000000.1
SI 2280/15	SC	Chicken carcass	Food	CFSAN107182	AAXHUK000000000.1
SI 2302/15	SC	Cleaning wipe	Environment	CFSAN107183	AAXHUC000000000.1
SI 2370/15	SC	Carcass cleaning wipe	Food	CFSAN107185	AAXHUH000000000.1
SI 2869/15	MG	Chicken upper leg	Food	CFSAN107190	AAXHUP000000000.1

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TABLE 1 (Continued)

	Isolation data			Accession no.	
Strain no.	**State**	**Material**	**Source**	**CFSAN Strain no.**	**GenBank accession no.**
SI 3056/15	MG	Chicken carcass	Food	CFSAN107193	AAXHUJ000000000.1
SI 4764/15	SC	Cleaning wipe	Environment	CFSAN107197	AAXHVH000000000.1
SI 5391/15	SC	Disposable shoe cover	Environment	CFSAN107200	AAXHUD000000000.1
SI 5837/15	SC	Disposable shoe cover	Environment	CFSAN107201	AAXHTN000000000.1
SI 5853/15	SC	Disposable shoe cover	Environment	CFSAN107202	AAXJLL000000000.1
SI 5859/15	SC	Disposable shoe cover	Environment	CFSAN107203	AAXHWB000000000.1
SI 5911/15	SC	Cleaning wipe	Environment	CFSAN107204	AAXHVK000000000.1
SI 5912/15	SC	Cleaning wipe	Environment	CFSAN107205	AAYKGL000000000.1
SI 5915/15	SC	Cleaning wipe	Environment	CFSAN107206	AAYKGJ000000000.1
SI 5923/15	SC	Cleaning wipe	Environment	CFSAN107207	AAYKGQ000000000.1
SI 220/16	SC	Cleaning wipe	Environment	CFSAN107212	AAYKGB000000000.1
SI 3687/16	SC	Chicken carcass	Food	CFSAN107222	AAYKGA000000000.1
SI 4447/16	SC	Pork sausage	Food	CFSAN107224	AAYKGC000000000.1
SI 5946/16	SC	Pork rib	Food	CFSAN107226	AAYAAA000000000.1
SI 6987/16	MA	Human faeces	Human	CFSAN107229	AAYAIC000000000.1
SI 7876/16	RS	Human faeces	Human	CFSAN107233	AAYAFO000000000.1
SI 11/17	PR	Dragging swab	Environment	CFSAN107235	AAYARD000000000.1
SI 23/17	PR	Dragging swab	Environment	CFSAN107237	AAYAFK000000000.1
SI 238/17	PR	Dragging swab	Environment	CFSAN107238	AAYAFN000000000.1
SI 872/17	MG	Chicken carcass	Food	CFSAN107239	AAYAFR000000000.1
SI 1171/17	SP	Soil	Environment	CFSAN107242	AAYAFL000000000.1
SI 1256/17	SP	Soil	Environment	CFSAN107243	AAYAFP000000000.1
SI 2580/17	SC	Human faeces	Human	CFSAN107259	AAYKFO000000000.1
SI 2953/17	GO	Human faecal swab	Human	CFSAN107261	AAYKFZ000000000.1
SI 2954/17	GO	Human faecal swab	Human	CFSAN107262	AAYKFE000000000.1
SI 3380/17	GO	Human faecal swab	Human	CFSAN107263	AAYKFP000000000.1
SI 3877/17	MG	Chicken wings	Food	CFSAN107264	AAYKFX000000000.1
SI 3906/17	SP	Sieve residue	Environment	CFSAN107265	AAYKFS000000000.1
SI 4065/17	PR	Human faeces	Human	CFSAN107266	AAYKFR000000000.1
SI 4067/17	PR	Human faeces	Human	CFSAN107267	AAYKGD000000000.1
SI 4069/17	PR	Human blood	Human	CFSAN107268	AAYKFD000000000.1
SI 52/18	MG	Chicken carcass	Food	CFSAN107270	AAYKFI000000000.1
SI 331/18	GO	Human faecal swab	Human	CFSAN107273	AAYKFT000000000.1
SI 623/18	SC	Human faeces	Human	CFSAN107279	AAYKFY000000000.1
SI 661/18	MS	Human faeces	Human	CFSAN107280	AAYKFW000000000.1
SI 942/18	RS	Human faecal swab	Human	CFSAN107281	AAYKFM000000000.1
SI 1634/18	SC	Yellowtail amberjack fish meat	Food	CFSAN107284	AAYKFQ000000000.1
SI 2676/18	GO	Avian reproductive matrix	Animal	CFSAN107285	AAYKFF000000000.1

Note: Cleaning wipe: material similar to synthetic tissues sold commercially for domestic cleaning; used on the isolation procedure of micro-organisms from industry and farm facility surfaces in Brazil.

Data also available in Vilela et al. Microbiol Resour Announc 10:e00313-21. https://doi.org/10.1128/MRA.00313-21.

AL, Alagoas; BA, Bahia; GO, Goiás; MA, Maranhão; MG, Minas Gerais; MS, Mato Grosso do Sul; PE, Pernambuco; PR, Paraná; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo.

Detection of antimicrobial resistance genes

The 80 strains studied were searched for the presence of antimicrobial resistance genes using a combined analysis of ResFinder 4.1 and AMRFinderPlus tools. ResFinder 4.1 is available at https://cge.cbs.dtu.dk/services/ResFinder/ (Bortolaia et al., 2020), and the search was conducted using a specific filter for *Salmonella* spp., a minimum threshold for the identity of 90% and a minimum length of 80%. AMRFinderPlus is an integrated tool of NCBI's Pathogen Detection system, available at https://www.ncbi.nlm.nih.gov/pathogens/isolates/.

Plasmid detection

The identification of plasmids and its respective incompatibility (Inc) groups were performed for the 80 *S*. Infantis strains studied using the PlasmidFinder 2.1 tool, available at https://cge.cbs.dtu.dk/services/PlasmidFinder/ (Carattoli et al., 2014). The search was conducted using the *Enterobacteriales* database, a minimum threshold for the identity of 90% and a minimum length of 80%.

RESULTS

Pulsed-field gel electrophoresis

The similarity dendrogram generated with PFGE results is presented in Figure 1. By PFGE the 80 S. Infantis studied were typed into 43 PFGE-types and allocated in three distinct clusters with a ≥78.2% similarity among them. PFGE-A grouped 27 strains (33.8%), which were isolated between 2014 and 2018 from food ($n = 13$), the environment ($n = 9$), humans ($n = 4$) and animal feed ($n = 1$) presenting ≥80.0% of genetic similarity. Two strains from this cluster were isolated from the state of São Paulo, while 25 strains were isolated from the three states (Paraná, Santa Catarina e Rio Grande do Sul) of the south region of Brazil. PFGE-B grouped 40 strains (50.0%) isolated from humans ($n = 14$), the environment ($n = 9$), food ($n = 9$), animals ($n = 5$) and animal feed ($n = 2$) between 2013 and 2018 from all Brazilian states cited on item 3.1 presenting ≥80.1% of genetic similarity. PFGE-C grouped 11 strains (13.8%), being four isolated from food in the state of Minas Gerais in 2014, four isolated from the environment in the state of Goiás in 2015, two isolated from animals in the state of Rio Grande do Sul in 2014 and one isolated from human in 2018 in the state of Goiás, presenting ≥87.3 of similarity. Strains SI 2385/13 and SI 4764/15 were allocated outside of the three main clusters detected (Figure 1). The discriminatory index (DI) of PFGE's dendrogram obtained for the strains studied was 0.966.

Antimicrobial susceptibility testing

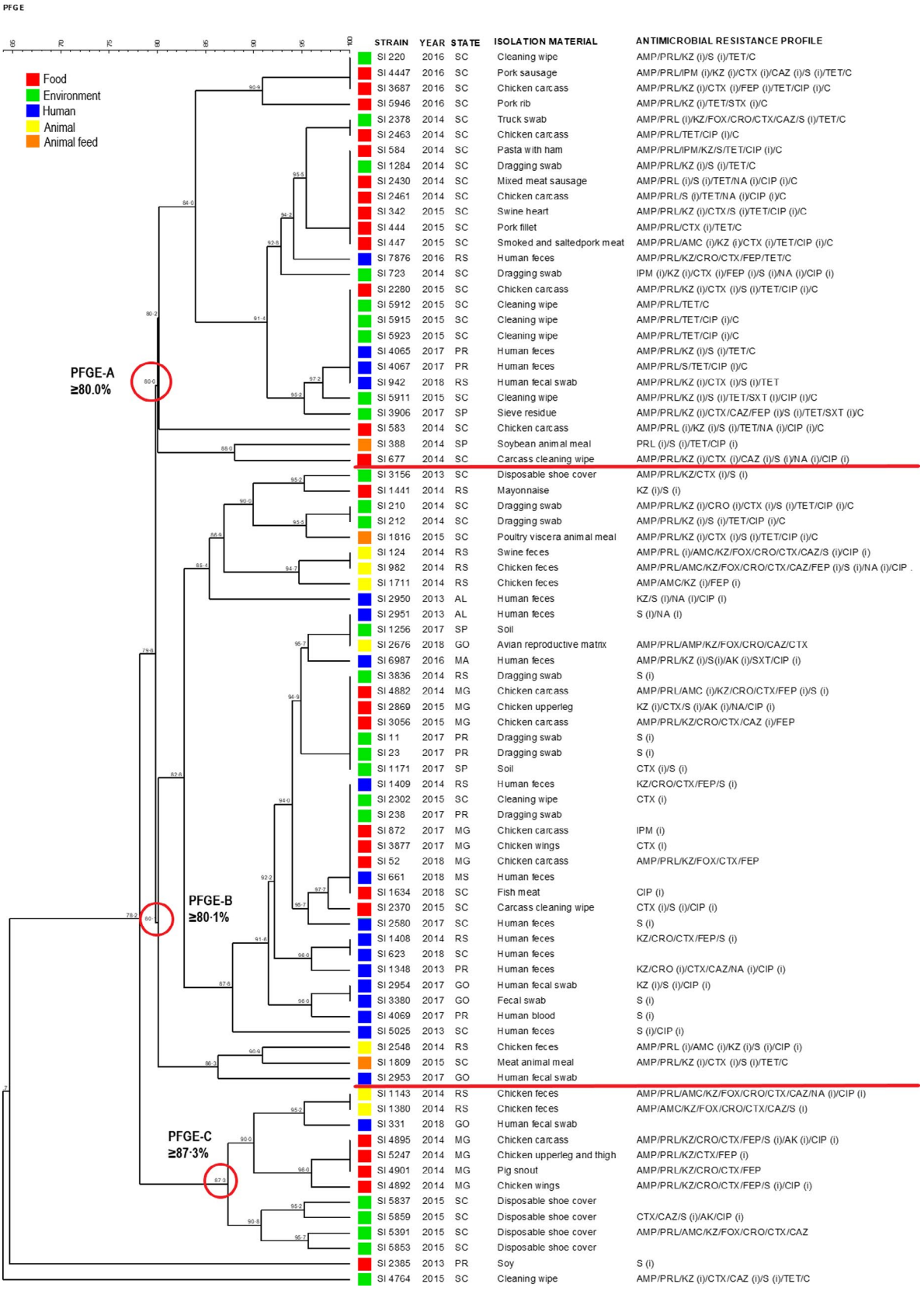
Resistant or intermediate resistance profiles to all antimicrobials tested were observed among the 80 *S*. Infantis strains studied. A total of 72 strains (90.0%) presented resistance or intermediate resistance to at least one of the antimicrobials tested, while eight (10.0%) were susceptible to antimicrobials tested. Moreover, 31 strains (38.8%) presented resistance to three or more antimicrobials of at least three different drug classes, showing a possible multidrug-resistant profile. Table 2 presents the percentages of resistant and intermediate resistant strains against the 18 antimicrobial agents tested. The specific phenotypic antimicrobial resistance profiles of each of the 80 *S*. Infantis strains studied are displayed in the Table S1.

MLST and SNP analyses

Regarding MLST, all the 80 *S*. Infantis strains studied were confirmed as belonging to the sequence type (ST) 32.

The phylogenetic tree generated with the SNP analysis conducted with CSI Phylogeny 1.4 is demonstrated in Figure 2, combined with the SNP clusters detected with NCBI's Pathogen Detection Isolate Browser and the antimicrobial resistance gene profiles (Figure 2).

FIGURE 1 Dendrogram representing genetic relationships among the 80 *Salmonella* Infantis strains studied isolated from food ($n = 27$), humans ($n = 19$), farm and industry environments ($n = 24$), animals ($n = 7$) and animal feed ($n = 3$) based on PFGE fingerprints. Similarity (%) between patterns was calculated using the DICE index and is represented by the numbers beside the nodes. The data were sorted by the UPGMA method. AL, Alagoas; GO, Goiás; MA, Maranhão; MS, Mato Grosso do Sul; MG, Minas Gerais; PR, Paraná; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo; (i), intermediate resistance profile. AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AK, amikacin; C, chloramphenicol; CAZ, ceftazidime; CIP, ciprofloxacin; CN, gentamycin; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitine; IPM, imipenem; KZ, cefazoline; NA, nalidixic acid; PRL, piperacillin; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline



The search of SNP clusters showed that 72 of the 80 *S.* Infantis strains studied were assigned to 13 different SNP clusters. A total of 48 strains were assigned into seven SNP clusters/profiles 1, 2, 8, 9, 10, 11 and 12 (accession numbers PDS000018462.71, PDS000029248.31, PDS000028532.2, PDS000074308.2, PDS000016779.4, PDS000026846.4

and PDS000075101, respectively) that also contained strains isolated from clinical and environmental/other sources from Brazil, Bolivia, Chile, Germany, Italy, United Kingdom and United States. Four strains were assigned into two SNP clusters/profiles 6 and 13 (PDS000020042.6 and PDS000078471.1 respectively) containing only additional isolates of environmental/other sources from Brazil. Finally, 20 strains were assigned into the four novel SNP clusters/profiles 3, 4, 5 and 7 (PDS000074994.3, PDS000074309.3, PDS000078491.1 and PDS000078459.1 respectively), that contained only strains from the present study. The distribution of strains into the 13 SNP clusters detect using NCBI's Pathogen Detection Isolate Browser is demonstrated in detail in Table 3. The individual SNP clusters of the 80 *S*. Infantis strains studied are also displayed in Figure 2.

Antimicrobial resistance genes

Through the combined analysis of ResFinder 4.1 and AMRFinderPlus, the *S*. Infantis strains studied harboured the β-lactam resistance genes $bla_{\text{TEM-1}}$ (40%), $bla_{\text{CTX-M-8}}$ (12.5%) and $bla_{\text{CMY-2}}$ (10.0%); diaminopyrimidine antibiotic resistance gene *dfrA8* (37.5%); tetracycline resistance gene *tet(A)* (36.3%); amphenicol resistance gene *floR* (36.3%); aminoglycoside resistance gene *aadA12* (2.5%); and sulfonamide resistance gene *sul2* (1.25%). Exclusively by ResFinder 4.1, strains also harboured the aminoglycoside gene *aac(6′)-Iaa* (100%), while by AMRFinderPlus, strains also showed the presence of the multidrug efflux pump coding genes *mdsA* (98.75%) and *mdsB* (98.75%) and aminoglycoside resistance genes *aph(3″)-Ib* (1.25%) and *aph(6)-Id* (1.25%).

TABLE 2 Number and percentage of resistant and intermediate resistant strains among the 80 *Salmonella* Infantis studied isolated from food ($n = 27$), humans ($n = 19$), farm and industry environments ($n = 24$), animals ($n = 7$) and animal feed ($n = 3$) in Brazil between 2013 and 2018

Antimicrobials	Resistant strains (%)	Intermediate resistant strains (%)
Penicillins		
Ampicillin	47 (57.8)	0
Piperacillin	41 (51.3)	6 (7.5)
β-lactam/β-lactamase inhibitor associations		
Amoxicillin - clavulanic acid	8 (10.0)	3 (3.8)
Carbapenems		
Imipenem	2 (2.5)	3 (3.8)
Cephalosporins		
Cefazolin	21 (26.3)	26 (32.5)
Cefoxitin	10 (12.5)	0
Ceftriaxone	19 (23.8)	2 (2.5)
Cefotaxime	24 (30.0)	16 (20.0)
Ceftazidime	11 (13.8)	4 (5.0)
Cefepime	9 (11.3)	7 (8.8)
Quinolones and Fluoroquinolones		
Nalidixic acid	4 (5.0)	10 (12.5)
Ciprofloxacin	1 (1.3)	34 (42.5)
Tetracyclines		
Tetracycline	30 (37.5)	0
Amphenicols		
Chloramphenicol	28 (35.0)	0
Aminoglycosides		
Streptomycin	2 (2.5)	48 (60.0)
Amikacin	2 (2.5)	3 (3.8)
Gentamicin	1 (1.3)	0
Sulphonamides		
Trimethoprim-sulfamethoxazole	2 (2.5)	3 (3.8)

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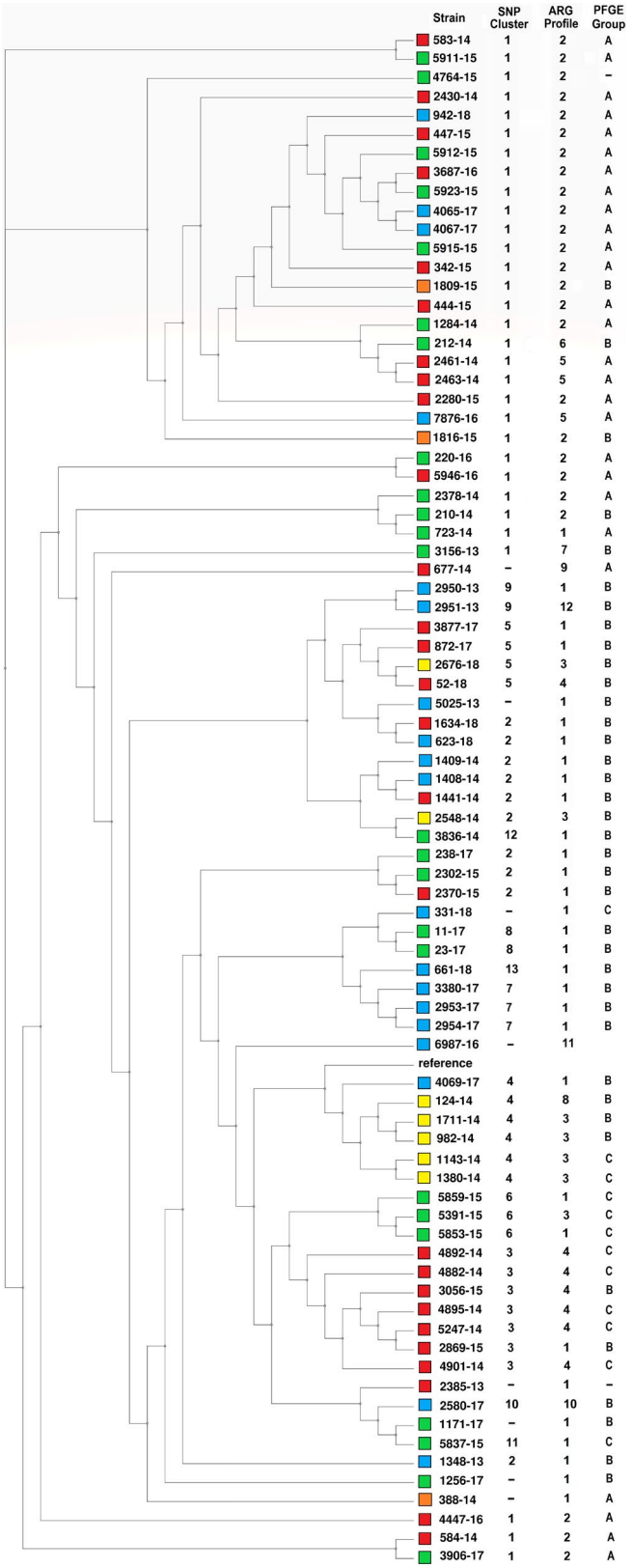

FIGURE 2 Phylogenetic tree based on the SNP analysis conducted with CSI Phylogeny 1.4 using the whole-genome sequences of the 80 *Salmonella* Infantis strains studied isolated from food (red squares; $n = 27$), farm and industry environments (green squares; $n = 24$), humans (blue squares; $n = 19$), animals (yellow squares; $n = 7$) and animal feed (orange squares; $n = 3$). *Salmonella* Infantis reference strain LN649235.1 was used for the alignment and included for comparison purposes in the phylogenetic tree. The number of the SNP clusters, detected using NCBI's Pathogen Detection Isolate Browser, are described in Table 3. The number of the antimicrobial resistance gene (ARG) profiles are described in Table 4. PFGE groups are marked in Figure 1

By the ResFinder 4.1 analysis, the strains studied also demonstrated to possess chromosomal point mutations in genes *gyrB* and *parC* of the quinolone determining region (QRDR), and in *pmrA* and *acrB* genes, which mediate resistance to antimicrobial peptides and multidrug efflux pumps respectively. In *gyrB*, all strains showed a point mutation from C to A in the codon that codifies the amino acid Glutamine (Gln) 624, leading to the formation of Lysin (Lys). In *parC*, all strains harboured a point mutation of C to G in the codon that codifies Threonine (Thr) 57 leading to the formation of Serine (Ser), and a second a point mutation from A to T on codon Thr255, leading to the formation of Serine (Ser). Strain SI 2580/17 also showed a *parC* point mutation from T to C in codon Valine (Val) 702, leading to the formation of Alanine (Ala). In *pmrA*, strain SI 124/14 displayed a point mutation from G to T in the codon Aspartic acid (Asp) 28, leading to the formation of Tyrosine (Tyr). In *acrB*, all strains showed a point mutation from T to C in codons Phenylalanine (Phe) 28 and in Leucine (Leu) 40, which led to the formation of Leu and Proline (Pro) respectively.

When combined, the content of acquired resistance genes and chromosomal point mutations detected among the 80 *S.* Infantis strains studied resulted in 11 genotypic antimicrobial resistance profiles, which are shown in Table 4. The genotypic profiles of each one of the 80 *S.* Infantis strains studied along the phenotypic profiles obtained are demonstrated in Table S1.

Plasmid content

Among the 80 *S.* Infantis strains studied, 24 (30.0%) harboured at least one type of plasmid. IncI1-I (Alpha) plasmids were the most frequent ones, being detected in 19 strains and related to the R64 plasmid of *Salmonella* Typhimurium (accession number AP005147.1). IncX1 plasmids were detected in three strains and showed to be correlated to pOLA52 plasmid of *Escherichia coli* (*E. coli*; accession number EU370913). IncFIB, IncFII and IncFII(29) were detected in individual strains and were similar to *E. coli* plasmids F (AP001918), pCA15-1A (AY458016) and pCE10A (CP003035) respectively. IncN (*Salmonella* Typhimurium plasmid R46; accession

TABLE 3 SNP clusters detected using NCBI's pathogen detection isolate browser among the 80 *Salmonella* Infantis strains studied isolated from food ($n = 27$), humans ($n = 19$), farm and industry environments ($n = 24$), animals ($n = 7$) and animal feed ($n = 3$) in Brazil between 2013 and 2018

SNP Profiles	No. of strains of this study	SNP Cluster accession no.	Additional global strains in SNP Cluster		Total no. of genomes
			Isolation material	Country of isolation	
1	31	PDS000018462.71	Clinical	United States ($n = 69$); No country informed ($n = 3$)	152
			Environmental/other	United States ($n = 43$); Brazil ($n = 6$)	
2	10	PDS000029248.31	Clinical	Bolivia ($n = 1$); Chile ($n = 1$); Germany ($n = 12$); Italy ($n = 2$); United Kingdom ($n = 32$); United States ($n = 4$)	87
			Environmental/other	Brazil ($n = 8$); Chile ($n = 5$); Germany ($n = 1$); United Kingdom ($n = 1$)	
			Not informed	Italy ($n = 2$)	
3	7	PDS000074994.3	—	—	7
4	6	PDS000074309.3	—	—	6
5	4	PDS000078491.1	—	—	4
6	3	PDS000020042.6	Environmental/other	Brazil ($n = 3$)	6
7	3	PDS000078459.1	—	—	3
8	2	PDS000028532.2	Clinical	United States ($n = 1$)	4
			Environmental/other	United Kingdom ($n = 1$)	
9	2	PDS000074308.2	Clinical	United Kingdom ($n = 1$)	4
			Environmental/other	Brazil ($n = 1$)	
10	1	PDS000016779.4	Clinical	United Kingdom (n = 3)	4
11	1	PDS000026846.4	Clinical	United Kingdom ($n = 1$)	5
			Environmental/other	Brazil ($n = 1$); United States ($n = 1$); United Kingdom ($n = 1$)	
12	1	PDS000075101.1	Clinical	United States ($n = 1$)	2
13	1	PDS000078471.1	Environmental/other	Brazil ($n = 1$)	2
—	8	Not detected	—	—	—

number AY046276), IncQ1 (*E. coli* RSF1010 plasmid; accession number M28829) and IncR (*Klebsiella pneumoniae* pK245; accession number DQ449578) were also detected in individual strains. The frequency of the plasmids detected are displayed in Table 4 along with the antimicrobial resistance profiles of each strain in Table S1.

DISCUSSION

Salmonella Infantis has been a highly prevalent serovar in many countries, capable to infect a broad range of food-producing animals as poultry, swine and bovine besides humans, and it has been also related to increasing antimicrobial resistance rates to drugs of clinical and veterinary relevance over recent years (Acar et al., 2019; Carfora et al., 2018; Ranjbar et al., 2018). In Brazil, despite the high detection of *S*. Infantis and the country's relevance in meat exportation, few studies have been conducted to better comprehend specific traits of the strains of this serovar circulating in the country, which may pose as a potential concern for food safety and public health fields (Almeida et al., 2013; Castro et al., 2002; Cunha-Neto et al., 2018; Fonseca et al., 2006; Monte et al., 2019; Moraes et al., 2000).

Over the years, molecular typing methods such as PFGE and MLST have been extensively and successfully used to subtype *Salmonella* serovars, including *S*. Infantis (Almeida et al., 2013; Kürekci et al., 2021; Mejía et al., 2020; Monte et al., 2019; Ranjbar et al., 2018; Xu et al., 2021). In addition, the advances achieved in WGS techniques in recent years allowed greater access to genomic-based typing methodologies, such as SNP analysis (Acar et al., 2019; Alba et al., 2020; Brown et al., 2018; Elbediwi et al.,

TABLE 4 Antimicrobial resistance gene (ARG) profiles of acquired resistance genes and chromosomal point mutations along with the plasmid profiles detected among the 80 *Salmonella* Infantis strains studied isolated from food ($n = 27$), humans ($n = 19$), farm and industry environments ($n = 24$), animals ($n = 7$) and animal feed ($n = 3$) in Brazil between 2013 and 2018

	Genotypic antimicrobial resistance profiles[a]		Isolation sources						
ARG profile	Acquired resistance genes[b]	Chromosomal point mutations	AN	FO	HU	EN	AF	Total	Associated plasmid profiles (no. strains)
1	*aac(6′)-Iaa, mdsA, mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	7	12	11	1	31	—
2	*aac(6′)-Iaa*, bla_{TEM-1}, *floR*, *dfrA8*, *tet(A)*, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	10	3	10	2	25	IncI1-I (Alpha), IncN and IncR ($n = 1$); IncQ1 ($n = 1$)
3	*aac(6′)-Iaa*, bla_{CMY-2}, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	6	—	—	1	—	7	IncI1-I (Alpha) ($n = 6$); IncI1-I (Alpha) and IncX1 ($n = 1$)
4	*aac(6′)-Iaa*, $bla_{CTX-M-8}$, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	7	—	—	—	7	IncI1-I (Alpha) ($n = 7$)
5	*aac(6′)-Iaa*, bla_{TEM-1}, $bla_{CTX-M-8}$, *floR*, *dfrA8*, *tet(A)*, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	2	1	—	—	3	IncI1-I (Alpha) ($n = 3$)
6	*aac(6′)-Iaa*, *aadA12*, bla_{TEM-1}, *floR*, *dfrA8*, *tet(A)*, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	—	—	1	—	1	IncX1 ($n = 1$)
7	*aac(6′)-Iaa*, *aadA12*, bla_{TEM-1}, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	—	—	1	—	1	IncX1 ($n = 1$)
8	*aac(6′)-Iaa*, bla_{CMY-2}, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro), *pmrA* (Asp28-Tyr)	1	—	—	—	—	1	IncI1-I (Alpha) ($n = 1$)
9	*aac(6′)-Iaa*, bla_{TEM-1}, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	1	—	—	—	1	IncFIB and IncFII ($n = 1$)
10	*aac(6′)-Iaa, mdsA, mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser, Val702-Ala), *acrB* (Phe28→Leu, Leu40→Pro)	—	—	1	—	—	1	—
11	*aph(3″)-Ib*, *aph(6)-Id*, *aac(6′)-Iaa*, bla_{TEM-1}, *dfrA8*, *sul2*, *mdsA*, *mdsB*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	—	1	—	—	1	IncFII(29) ($n = 1$)
12	*aac(6′)-Iaa*	*gyrB* (Gln624→Lys), *parC* (Thr57→Ser, Thr255→Ser), *acrB* (Phe28→Leu, Leu40→Pro)	—	—	1	—	—	1	—

AF, animal feed; AN, animal; EN, environment; FO, food; HU, human.

[a]Acquired resistance genes were detected using a combined analysis of ResFinder 4.1 and AMRFinderPlus v. 3.3.28.

[b]*aac(6′)-Iaa* was only detected using ResFinder 4.1. *mdsA*, *mdsB*, *aph(3″)-Ib* and *aph(6)-Id* were only detected using AMRFinderPlus v. 3.3.28.

2021). However, to date, few studies have been conducted in Brazil using PFGE and MLST to exclusively characterize a great number of *S*. Infantis strains and none have employed genomic methods such as SNP analysis.

In the present study, PFGE demonstrated the presence of three clusters, respectively containing 33.8%, 50.0% and 13.8% of the 80 *S*. Infantis strains analysed (Figure 1). No clear correlation was observed within the clusters

regarding the sources, material, years and states of isolation and/or the antimicrobial resistance profiles of the strains (Figure 1), which suggested that *S*. Infantis strains from these clusters may have been circulating in the country over different states of Brazil, promoting a possible transmission among food sources, humans, the environment and veterinary-related sources.

Previous studies conducted in Brazil used PFGE to characterize *S*. Infantis strains isolated from human faeces and food sources over a 25-year period in the state of São Paulo (Almeida et al., 2013) and blood, faeces and cerebrospinal fluid isolates from adults and newborn children in public hospitals of the city of Rio de Janeiro (Fonseca et al., 2006; Pessoa-Silva et al., 2002), and reported a high genetic similarity among isolates of this serovar. In other countries, several studies also analysed *S*. Infantis genotypic diversity by PFGE. In Papadoupoulos et al. (2017), 40 strains of this serovar isolated from humans, food and animals in Greece from 2007 to 2010 were grouped in 31 PFGE-types, four groups with an overall similarity of ≥87% and a DI of 0.965 (Papadopoulos et al., 2017). Rahmani et al. (2013) reported the presence of only two PFGE-types among 27 *S*. Infantis strains isolated from chickens between 2007 and 2011, with 26 strains indistinguishable according to PFGE (Rahmani et al., 2013).

While PFGE showed the presence of three major clusters, the SNP analyses conducted divided 72 of the 80 strains studied into 13 SNP clusters/profiles (Table 3; Figure 2). The most frequent profile detected was SNP cluster 1, that was assigned to 31 of the 80 *S*. Infantis strains studied (38.8%), which were isolated between 2013 and 2018 from food, environmental, human and animal feed sources (Figure 2; Table 3). Despite of the clear difference in the discriminatory capacity, PFGE showed to be concordant with the SNP analyses. This fact was clearly observed because the major part of the strains from SNP cluster/profile 1 were also located into the PFGE-A group (Figure 2). Moreover, while PFGE-B group was initially demonstrated to be the one containing the higher number of strains, when combined to the SNP analysis, it was clearly demonstrated its association with strains of diverse SNP clusters/profiles (Figure 2). Together, the results of PFGE and the SNP analyses suggested the possible presence of a prevalent *S*. Infantis subtype (PFGE-A and SNP cluster/profile 1) and its possible transmission among food sources, humans, the environment and veterinary sources in various states of Brazil.

Among the 13 SNP clusters detected, 48 of the 80 *S*. Infantis strains studied (60.0%) were assigned into seven SNP clusters/profiles (1, 2, 8, 9, 10, 11 and 12) that also contained isolates deposited in NCBI's Pathogen Detection from Brazil, Bolivia, Chile, Germany, Italy, United Kingdom and United States (Figure 2; Table 3). These data demonstrated that most part of *S*. Infantis strains circulating in Brazil are not only spread among different sources and states inside the country, but were also genetically related to international *S*. Infantis strains, suggesting a possible international dissemination of some subtypes of this serovar.

However, it is also worth to notice that, in the present study, 32 of the 80 *S*. Infantis strains studied (40.0%) were assigned into SNP clusters/profiles containing exclusively Brazilian isolates (Figure 2; Table 3). Four strains were assigned into SNP profiles 6 and 13 that already contained *S*. Infantis strains previously deposited into NCBI's Pathogen Detection database (Table 3). The novel SNP profiles 3, 4, 5 and 7 contained exclusively 20 *S*. Infantis strains (25.0%) that were analysed here in this study. Finally, eight strains were not assigned into any SNP cluster in the database (Figure 2; Table 3), suggesting that these isolates possess unique profiles that have not been yet associated to any other strain. Together, considering the role of Brazil as a leading meat exporter, these results also reinforced the necessity of stronger control measures to prevent the dissemination of novel *S*. Infantis subtypes in Brazil and other countries.

To the best of our knowledge, no study conducted in Brazil exclusively characterized and compared a great number of *S*. Infantis strains using SNP-based analyses using WGS data. However, reports from other countries have already employed this methodology to subtype strains of this serovar. Brown et al. (2018) identified the presence of two major groups, according to the SNP analysis, among 34 strains isolated from humans and one from chicken meat between 2012 and 2015 in the United States, and that the major group reported contained 32 strains with a high genetic relatedness (Brown et al., 2018). In Acar et al. (2019), 23 *S*. Infantis strains obtained from chicken meat in Turkey from 2012 to 2013 were allocated into a single cluster presenting a high genetic correlation in comparison with 234 *S*. Infantis genomes from nine different countries (Acar et al., 2019). Alba et al. (2020) reported the presence of nine distinct clusters by the SNP analysis when comparing 382 strains isolated from diverse sources from nine European countries (Alba et al., 2020).

Through MLST, all the *S*. Infantis strains analysed in the present study were typed as belonging to ST32. Similarly, previous studies conducted in Brazil and in other countries have also demonstrated the high prevalence of this ST among strains of this serovar, indicating its global predominance for *S*. Infantis strains (Almeida et al., 2013; Mejía et al., 2020; Monte et al., 2019; Ranjbar et al., 2018). However, different STs have also been detected in reduced rates for *S*. Infantis strains isolated in other countries, such as ST2283 and ST1032 among strains isolated

from chickens in Germany from 1995 and 1996 and from 2014 and 2019 (García-Soto et al., 2020) and ST7091 in a single *S*. Infantis strain isolated in 2017 from chicken in Turkey (Kürekci et al., 2021). Although MLST did not provide an adequate capacity to allow the differentiation of *S*. Infantis strains from Brazil as PFGE and SNP analysis, the dominance of strains from ST32 demonstrated the capacity of this methodology to identify strains of this serovar into specific STs.

Over recent years, *S*. Infantis strains also demonstrated an alarming increase in antimicrobial resistance rates for drugs of clinical and veterinary use, which poses as a concern for public health and food safety authorities due to the broad distribution of this serovar, its ability to infect a wide range of hosts and the possibility of transmission of drug-resistant *S*. Infantis strains to humans by the consumption of contaminated food (Acar et al., 2019; Brown et al., 2018; Carfora et al., 2018; Cunha-Neto et al., 2018; Kalaba et al., 2017; Ranjbar et al., 2018).

Part of the group of β-lactams antimicrobial drugs, third and fourth-generation cephalosporins are one of the classes of agents considered as 'drugs of choice' for the treatment of serious *Salmonella* infections in humans (Christenson, 2013; McDermott et al., 2018). In the present study, the 80 *S*. Infantis strains studied showed phenotypic resistance rates ranging from 2.5% to 57.8% for β-lactams antimicrobials, including third- and fourth-generation cephalosporins, which rates ranged from 11.3% to 30% (Table 1). The search for resistance genes through WGS showed that the strains studied harboured β-lactam resistance genes $bla_{\text{TEM-1}}$ in 40% of the strains, $bla_{\text{CTX-M-8}}$ in 12.5% and $bla_{\text{CMY-2}}$ in 10.0% (Table 4). Previous studies also reported similar phenotypic profiles associated to the presence of the same resistance genes among *S*. Infantis strains isolated in Brazil and other countries (Fonseca et al., 2006; Monte et al., 2019; Moraes et al., 2000; Shahada et al., 2010; Vilela et al., 2020).

It is interesting to notice that, when the genotypic resistance profiles were compared to the SNP profiles/clusters detected (Figure 2), the strains harbouring the β-lactam resistance gene $bla_{\text{TEM-1}}$ were predominant in the SNP cluster/profile 1, which was also demonstrated to be the main SNP cluster among the *S*. Infantis strains studied and to be genetically associated with strains also isolated in the United States (Figure 2; Tables 3 and 4). Strains harbouring β-lactam resistance genes $bla_{\text{CTX-M-8}}$ and $bla_{\text{CMY-2}}$ showed to be more associated to SNP clusters/profiles 3 and 4, respectively, which were more correlated to strains of this serovar exclusively isolated in Brazil (Figure 2; Tables 3 and 4). These results highlight the possible potential of WGS to identify regional and global subtypes of relevant zoonotic and foodborne pathogens, such as *S*. Infantis, and to monitor the dissemination of important antibiotic resistance genes.

The second class of antimicrobial agents recommended for the treatment of severe *Salmonella* infections in humans are fluoroquinolones, such as ciprofloxacin, which are derivatives from quinolones drugs, such as nalidixic acid (Aldred et al., 2014; Hawkey, 2003). Among the *S*. Infantis strains studied, low phenotypic resistance rates to both nalidixic acid and ciprofloxacin were noticed (Table 2), as well as the absence of acquired resistance genes for this drug class. However, more than 40% of the strains presented phenotypic intermediate resistance profiles to ciprofloxacin (Table 2) and all strains showed QRDR point mutations in *gyrB* (Gln624→Lys) and *parC* (Thr57→Ser e Thr255→Ser) (Table 4; Table S1).

Point mutations in the QRDR genes are well known to result in different resistance levels to quinolones and fluoroquinolones, and *gyrA* mutations are generally the main responsible for high levels of resistance for this drug among *Salmonella* serovars. In previous reports, Asp87→Tyr in *gyrA* was the main mutation associated with increased levels of resistance in *S*. Infantis strains (Nakatsuchi et al., 2018; Velhner et al., 2014). Mutation Thr57→Ser in *parC*, which was detected in all the *S*. Infantis strains in the present study, has been described in *Salmonella* strains resulting in low levels of resistance for ciprofloxacin (Eaves et al., 2004), which corroborates with the low rates of phenotypic resistance to quinolones observed in the present results. However, the point mutations Gln624→Lys in *gyrB* and Thr255→Ser and Val702→Ala in *parC*, that were also detected in this study, have not been reported and described yet in *Salmonella* strains, which difficult the understanding of the quinolone and fluoroquinolone resistance levels conferred by these chromosomal mutations and, therefore, reinforced the necessity of further studies.

The results obtained suggested that the increasing levels of β-lactam resistance and intermediate levels of ciprofloxacin resistance, which could be noticed by phenotypic and genotypic methods, should be an alert for the necessity to monitor *S*. Infantis due to the potential selection of strains presenting antimicrobial resistance profiles to agents considered as first choice for the treatment of human infections and its possible transmission by the consumption of contaminated food.

It is worth to notice that a significant number of strains in the present study showed resistance to antimicrobials agents that are not currently used for human therapy of *Salmonella* infections but are still broadly used in the veterinary field. Acquired resistance genes such as *tet(A)*, *floR*, *dfrA8*, *sul2*, *aadA12*, *aph(3″)-Ib* and *aph(6)-Id* (Tables 2 and 4, Table S1), have been previously reported to be broadly distributed and to promote

phenotypic resistance to drugs as tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and aminoglycosides among *Salmonella* serovars such as *S.* Infantis strains from Brazil and other countries (Brown et al., 2018; Cunha-Neto et al., 2018; Elbediwi et al., 2021; Ranjbar et al., 2018; Shahada et al., 2010; Xu et al., 2021). In contrast, cryptic genes such as the aminoglycoside resistance gene *aac(6)-Iaa* are highly frequent among *Salmonella* serovars, but are not capable to provide phenotypic resistance in these strains (Magnet et al., 1999). These resistance profiles may not only occur as a result of the large use that these agents had in the past in human therapy, but also due to its broad and sometimes indiscriminate application in Brazil as prophylactics or growth promoters in the production of farm animals such as poultry, swine and bovines, which may influence the dissemination of drug-resistant *S.* Infantis through food sources (McDermott et al., 2018; Xiong et al., 2018).

Moreover, it is also interesting to point that the *S.* Infantis strains studied harboured multidrug efflux pump coding genes, such as *msdA* and *mdsB* in 98.75% of the strains studied, as well as *acrB* point mutations Phe28→Leu and Leu40→Pro in all strains (Table 4, Table S1). In contrary to the other genes detected among the *S.* Infantis strains studied, which are usually antibiotic-specific, efflux pump coding genes such as *mdsAB* and *acrB* do not present this specificity, but are capable to lead to resistance against multiple classes of antimicrobial agents, such as β-lactams, fluoroquinolones, tetracyclines, amphenicols, glycylcyclines, rifamycin and biocide agents (Pontel et al., 2007; Song et al., 2014). In this way, the presence of these above-mentioned genes and point mutations among *S.* Infantis strains may suggest that other genetic mechanisms, capable to provide unspecific and broader resistance to multiple agents, may also contribute to the increasing antimicrobial resistance rates in strains of this serovar to drugs of clinical and non-clinical use.

Plasmids have been demonstrated to play a relevant role into the acquisition of different antimicrobial resistance genes among *Salmonella* serovars, including *S.* Infantis (Alba et al., 2020; Carattoli et al., 2014; Kürekci et al., 2021; McDermott et al., 2018). In the present study, 24 of the 80 *S.* Infantis strains analysed (30.0%) harboured at least one plasmid type. These results, together with the fact that all strains analysed harboured at least one type of resistance gene (as mentioned above), suggested that the major part of resistance genes detected in the *S.* Infantis here analysed possessed a chromosomal location instead of a plasmid origin.

The IncI1-I (Alpha) plasmid, identified as the *Salmonella* Typhimurium R64 plasmid, was the most frequently detected, in 19 of the 80 strains analysed. This plasmid has been first described as tetracycline and streptomycin resistance plasmid (Sampei et al., 2010), but in the present study, this was mostly detected in strains harbouring $bla_{\text{CTX-M-8}}$ and $bla_{\text{CMY-2}}$ (Table 4; Table S1), which has been similarly reported in previous studies with diverse *Salmonella* serotypes presenting variants of these same genes (Kameyama et al., 2012; Tiba-Casas et al., 2019).

Seven strains with $bla_{\text{TEM-1}}$ and other non-beta-lactam resistance genes also harboured IncI1-I (Alpha), IncX1, IncF, IncN, IncQ and IncR plasmids (Table 4; Table S1). However, since most of these genes were detected even in higher frequencies among strains harbouring no plasmids, it may suggest that among the *S.* Infantis here studied, such resistance genes may be located into the bacterial chromosome instead of inside these plasmids, and that these mobile elements could also be associated to other functions besides antimicrobial resistance, such as virulence or environmental adaptation. It is also important to notice that additional studies should be performed to provide detailed information and a deeper overview of the genetic structure of these plasmids.

Finally, it should be stated that it was not possible to observe a complete correlation between the detection of resistance genes using WGS data and the phenotypic antimicrobial resistance rates observed by disk-diffusion among all strains analysed (Tables 2 and 4, Table S1). As previous studies conducted with *Salmonella* serovars have already reported (Almeida et al., 2018; Campioni et al., 2020; McDermott et al., 2016), this fact is probably related to the presence of resistance mechanisms not yet discovered or included in the databases of searching platforms, which reinforced the necessity of constant monitoring of the emergence of novel antimicrobial resistance traits.

In conclusion, PFGE and SNP analysis exhibited similar results and suggested the presence of a prevalent *S.* Infantis subtype circulating among different sources and regions of Brazil, while MLST reinforced the dominance of ST32 in the strains studied. The high rates of phenotypic and genotypic resistance to antimicrobial agents used in the treatment of infections in humans and in the veterinary field alerted for the potential risk of transmission of drug-resistant *S.* Infantis strains to humans by the consumption of contaminated food. Moreover, the relative small frequency of plasmids among the strains studied suggested that the high frequency of antimicrobial resistance genes detected may have a chromosomal location. Together, the results obtained reinforced the potential hazard that *S.* Infantis strains may represent for the public health and food safety fields in Brazil and other countries.

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CONFLICT OF INTEREST

No conflict of interest declared.

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