Phylogenetic and antimicrobial resistance gene analysis of *Salmonella* Typhimurium strains isolated in Brazil by whole genome sequencing

Fernanda Almeida\(^1\)\(^*,\) Amanda Aparecida Seribelli\(^1\)\(^*,\) Marta Inês Cazentini Medeiros\(^2\), Dália dos Prazeres Rodrigues\(^3\), Alessandro de Mello Varani\(^4\), Yan Luo\(^5\), Marc W. Allard\(^5\)\(^*\), Juliana Pfrimer Falcão\(^1\)\(^*\) 

\(^1\) Departamento de Análises Clínicas, Toxicológicas e Bromatológicas—Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, Ribeirão Preto, SP Brasil, \(^2\) Centro de Laboratório Regional de Ribeirão Preto—Instituto Adolfo Lutz, Rua Minas, Ribeirão Preto, SP, Brasil, \(^3\) Laboratório de Enterobactérias, FIOCRUZ/Fundação Instituto Oswaldo Cruz, Avenida Brasil, Pavilhão Rocha Lima, 3° andar, Manguinhos, Rio de Janeiro, RJ, Brasil, \(^4\) Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal, Brazil, \(^5\) Division of Microbiology, Office of Regular Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland, United States of America

\(^*\) These authors contributed equally to this work.
\(^\dagger\) First authors.

\(^*\)jufalcao@fcfrp.usp.br (JPF); marc.allard@fda.hhs.gov (MA)

**Abstract**

Whole genome sequencing (WGS) has been used as a powerful technology for molecular epidemiology, surveillance, identification of species and serotype, identification of the sources of outbreaks, among other purposes. In Brazil, there is relatively few epidemiological data on *Salmonella*. In this study, 90 *Salmonella* Typhimurium strains had their genome sequenced to uncover the diversity of *Salmonella* Typhimurium isolated from humans and food, between 1983 and 2013, from different geographic regions in Brazil based on single nucleotide polymorphism (SNP) analysis. A total of 39 resistance genes were identified, such as aminoglycoside, tetracycline, sulfonamide, trimethoprim, beta-lactam, fluoroquinolone, phenicol and macrolide, as well as the occurrence of point mutations in some of the genes such as *gyrA*, *gyrB*, *parC* and *parE*. A total of 65 (72.2%) out of 90 *S*. Typhimurium strains studied were phenotypically resistant to sulfonamides, 44 (48.9%) strains were streptomycin resistant, 27 (30%) strains were resistant to tetracycline, 21 (23.3%) strains were gentamicin resistant, and seven (7.8%) strains were resistant to ceftriaxone. In the *gyrA* gene, it was observed the following amino acid substitutions: Asp(87)→Gly, Asp(87)→Asn, Ser(83)→Phe, Ser(83)→Tyr. Phylogenetic results placed the 90 *S*. Typhimurium strains into two major clades suggesting the existence of a prevalent subtype, likely more adapted, among strains isolated from humans, with some diversity in subtypes in foods. The variety and prevalence of resistant genes found in these *Salmonella* Typhimurium strains reinforces their potential hazard for humans and the risk in foods in Brazil.
Introduction

Foodborne diseases have a major impact on the economy and public health worldwide. Non-typhoidal Salmonella (NTS) is one of the most common causes of bacterial foodborne illnesses [1, 2]. It is estimated that NTS cause about 93.8 million annual cases of gastroenteritis and 155 thousand deaths per year worldwide [1].

Among the Salmonella enterica serovars, Salmonella Typhimurium (S. Typhimurium) is one of the most frequent ones isolated worldwide [3]. From 2001 to 2007, this serovar was the most prevalent in the United States, Canada, Australia and New Zealand. In the same period, S. Typhimurium appeared as the second most prevalent serovar in Africa, Asia, Europe and Latin America, surpassed only by S. Enteritidis [3].

In Brazil, there are relatively little epidemiological data on Salmonella [4–7]. However, it is known that in the State of São Paulo, S. Typhimurium was the most commonly isolated serovar from human sources and the third most common from non-human sources before the 1990’s [4]. After this period, S. Typhimurium declined becoming the third most commonly isolated serovar from human and non-human sources in the period of 1991–1995 in São Paulo State in Brazil, with S. Enteritidis being the most isolated serovar in both sources and, S. I 4, (5), 12:i-: and S. Havana the second most isolated serovar in human and non-human sources, respectively [5]. Between 1996 and 2000, the isolation of S. Typhimurium declined even more from non-human sources [6]. However, between 1996 and 2003, this serovar was ranked as the second most commonly isolated serovar from human sources [7].

Epidemiological studies have been crucial to verify the relationship among pathogenic strains isolated from different sources, to elucidate contamination routes and to differentiate strains isolated from outbreaks and sporadic cases. Investigative capabilities have been greatly enhanced with the development and increasing feasibility of WGS as a molecular epidemiological tool [8–10]. Over the last few years there has been a substantial reduction in the costs of WGS making this technology economically viable as a routine tool for molecular epidemiology. WGS has also been used for detection of antibiotic resistance determinants [11, 12].

The use of antimicrobials is not recommended in cases of noninvasive Salmonella infections [13, 14]. However, in some cases, the antibiotic therapy might be necessary. The drug of choice for the treatment of Salmonella infections is typically ciprofloxacin due to its broad spectrum antimicrobial activity [14].

The extensive use of antimicrobials has led to increasing numbers of non-typhoidal Salmonella strains that are resistant to quinolones and exhibited reduced susceptibility to fluoroquinolones [15–17]. This reduced susceptibility can lead to treatment failures in some cases [18, 19]. Quinolone resistance is usually mediated by mutations in the quinolone resistance determining regions (QRDRs) of the gyrA, gyrB, parC, and parE genes that code for bacterial DNA gyrase leading to changes in the binding site of the antimicrobial to the enzyme [17, 20, 21]. Also, quinolone resistance may be due to the acquisition of plasmid-mediated quinolone resistance (PMQR) genes [22–24], such as the qnr genes that encode a group of pentapeptide proteins that bind to DNA gyrase and prevent the action of quinolones, qepA gene, an quinolone efflux pump, aac(6’)-Ib-cr gene that encodes to the aminoglycoside acetiltransferase that can reduce susceptibility to ciprofloxacin and oqxAB genes, a multidrug resistance efflux pump [25].

In previous studies of our group, we typed S. Typhimurium strains isolated from humans and food between 1983 and 2013 in Brazil by Pulsed-field gel electrophoresis (PFGE), multiple-locus variable number of tandem repeats analysis (MLVA), enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), CRISPR-multi-locus virulence sequence typing (CRISPR-MVLST) and Multilocus sequence typing (MLST). Moreover, the frequency of 12
virulence markers was assessed by PCR and the resistance profile against 12 antimicrobials was verified [26–28].

In this present work, WGS is used to uncover the diversity of Salmonella Typhimurium isolated from humans and food, between 1983 and 2013, from different geographic regions in Brazil. Additionally, WGS is used to verify the presence of antimicrobial resistance genes, as well as, the occurrence of mutations points in the \( \text{gyrA}, \text{gyrB}, \text{parC} \) and \( \text{parE} \) genes.

**Materials and methods**

**Bacterial strains**

A total of 90 S. Typhimurium strains were sequenced including: 42 strains from human clinical material such as diarrheic feces (n = 40), blood (n = 1) and brain abscess (n = 1) between 1983 and 2010; and 48 strains from food such as chicken (n = 8), poultry (n = 3), swine (n = 11), meats (n = 23), vegetables (n = 2) and unknown (n = 1). Samples were collected between 1999 and 2013 from seven States of Brazil including: São Paulo; Santa Catarina; Paraná; Mato Grosso do Sul; Rio Grande do Sul; Goiás; and Bahia (Table 1). Strains were provided by Adolf Lutz Institute of Ribeirao Preto and Oswaldo Cruz Foundation (FIOCRUZ).

**DNA extraction and quantification**

The genomic DNA extraction methods followed Campioni and Falcão [29]. The quality of the DNAs were checked using NanoDrop 1000 (Thermo Scientific, Rockford, IL), and the concentrations were determined using Qubit double-stranded DNA BR assay kit and Qubit fluorometer (Life Technologies, Grand Island, NY) according to each manufacturer’s instructions.

**Genome sequencing, assembly, and annotation**

All isolates were prepared using the Nextera Sample Preparation Kit (Illumina, San Diego, CA) and then sequenced on Illumina NextSeq (Illumina) for 2 x 151 cycles. \( \text{De novo} \) assemblies were generated from all raw sequence data. The Illumina reads were assembled with SPAdes 3.0 with the following parameters: only contigs of length \( \geq 500 \) bp were included; mismatch (MM) 3.28; the genome fraction was 96.157; and number of mis-assemblies (MA) was 2 [30]. The contigs for each isolate (draft genome) were annotated using NCBI’s Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) [31]. The draft genome sequences of S. Typhimurium strains are publicly available in GenBank, with accession numbers listed in S1 Table. The presence of resistance genes, as well as points mutation in the QRDR of the \( \text{gyrA}, \text{gyrB}, \text{parC} \), and \( \text{parE} \) genes, were determined using ResFinder (Center for Genomic Epidemiology, https://cge.cbs.dtu.dk/services/ResFinder/) with settings of threshold of 90%, and minimum length of 60% [32].

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility of the 90 S. Typhimurium strains were tested by the disc diffusion method of the Clinical and Laboratory Standards Institute (CLSI) [33]. The majority of these results were previously published in Almeida et al. (2015) for 12 antimicrobials including: cefotaxime; cefoxitin; ceftazidime; aztreonam; cefepime; amoxicillin-clavulanic acid; ampicillin; nalidixic acid; levofloxacin; trimethoprim-sulfamethoxazole; chloramphenicol; and ciprofloxacin (Oxoid). However, five additional antimicrobials were tested in this study including: gentamicin; streptomycin; tetracycline; sulfonamides; and ceftriaxone. Additionally, the minimum inhibitory concentrations (MIC) of fluoroquinolones in the nalidixic acid resistant and susceptible strains were evaluated using Etest® following the Clinical and Laboratory Standards
Table 1. Phenotypic and genotypic resistance profiles of the 90 *Salmonella* Typhimurium strains studied isolated from humans and food in various States between 1983 and 2013 in Brazil.

| CFSA N° | Isolate name | Source | State | Year of isolation | Phenotypic Resistance Profile | Genotypic Resistance Profile (Identity %) | Aminoglycoside | Tetracycline | Sulphonamide | Trimethoprim | Beta-lactum | Fluoroquinolone | Phenicol |
|---------|--------------|--------|-------|------------------|-------------------------------|------------------------------------------|----------------|--------------|--------------|--------------|-------------|----------------|----------|
| CFSAN033848 | STm01 | Human feces | SP | 1983 | AMP-NA-SXT-STR |aadA1 (100), aph(3')-Ia (99.57) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033849 | STm02 | Human feces | SP | 1983 | AMC-AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aph(3')-Ia (99.96), dfrA1 (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033850 | STm03 | Human feces | SP | 1983 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aph(3')-Ia (99.39), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033851 | STm04 | Human feces | SP | 1983 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aph(3')-Ia (99.39), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033852 | STm05 | Human feces | SP | 1983 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aph(3')-Ia (99.39), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033853 | STm06 | Human feces | SP | 1983 |  |  |  |  |  |  |  |  |  |
| CFSAN033854 | STm07 | Human feces | SP | 1983 | AMP-NA-SXT-C-STR |aph(3')-Ia (99.25), aadA1 (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033855 | STm09 | Human feces | SP | 1984 | AMP-NA-SXT-C-GEN-SUL |aadA1 (100), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033857 | STm10 | Human feces | SP | 1984 | NA-SXT-SUL |  |  |  |  |  |  |  |  |
| CFSAN033858 | STm11 | Human feces | SP | 1984 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033859 | STm12 | Human feces | SP | 1984 | NA-GEN-STR-SUL |aacA4 (99.64), aadA1 (100), aph(3')-Ia (99.39) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033860 | STm13 | Human feces | SP | 1984 | AMP-NA-SXT-C-GEN-SUL |aadA1 (100), ant2'-Ia (99.06) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033861 | STm14 | Human feces | SP | 1984 | AMP-NA-SXT |aph(3')-Ia (99.39) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033862 | STm15 | Human feces | SP | 1985 | SUL |  |  |  |  |  |  |  |  |
| CFSAN033863 | STm16 | Human feces | SP | 1985 | NA-SXT |aadA1 (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033864 | STm17 | Human feces | SP | 1985 |  |  |  |  |  |  |  |  |  |
| CFSAN033865 | STm18 | Human feces | SP | 1985 |  |  |  |  |  |  |  |  |  |
| CFSAN033866 | STm19 | Human feces | SP | 1986 | AMP-NA-SXT-C-GEN-STR-SUL-CRO |aacA4 (99.66), aadA1 (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033867 | STm20 | Human feces | SP | 1986 | NA-SXT-C-TET-STR |aadA1 (100), aph(3')-Ia (99.39) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033868 | STm21 | Human feces | SP | 1986 | NA-STR |aadA1 (100), aph(3')-Ia (99.27) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033869 | STm22 | Human feces | SP | 1986 | AMC-AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aacA4 (99.64) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033870 | STm23 | Human feces | SP | 1986 | TET-STR |strA (100), aph(6)-Id (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033871 | STm24 | Human feces | SP | 1986 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aacA4 (99.64) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033872 | STm25 | Human feces | SP | 1986 | AMP-NA |aadA1 (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033873 | STm26 | Human feces | SP | 1986 | NA-STR |aadA1 (100), aph(3')-Ia (99.47) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033874 | STm27 | Human feces | SP | 1986 | AMP-NA-SXT-C-GEN-STR-SUL |aadA1 (100), aacA4 (99.64) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033875 | STm28 | Human feces | SP | 1988 | SUL |  |  |  |  |  |  |  |  |
| CFSAN033876 | STm29 | Human feces | SP | 1989 | AMP-SUL |aph(6)-Id (100), aph(3')-B (100) | tet(C) (99.92) | sul (100) | dfrA1 (100) |  |  |  |  |
| CFSAN033877 | STm30 | Human feces | SP | 1990 | SUL |  |  |  |  |  |  |  |  |
| CFSAN033878 | STm31 | Human feces | SP | 1991 | SUL |  |  |  |  |  |  |  |  |
| CFSAN033879 | STm32 | Human feces | SP | 1992 | SUL |  |  |  |  |  |  |  |  |
| CFSAN033880 | STm33 | Human feces | SP | 1992 |  |  |  |  |  |  |  |  |  |
| CFSAN033881 | STm34 | Human feces | SP | 1993 |  |  |  |  |  |  |  |  |  |

(Continued)
| CFSAN n° | Isolate name | Source | State | Year of isolation | Phenotypic Resistance Profile | Genotypic Resistance Profile (Identity %) |
|----------|--------------|--------|-------|-------------------|------------------------------|----------------------------------------|
|          |              |        |       |                   | Aminoglycoside | Tetracycline | Sulfonamide | Trimethoprim | Beta lactam | Fluoroquinolone | Phenicol |
| CFSAN033882 | STm35 | Human feces | SP | 1995 | SUL | — | — | — | — | — | — |
| CFSAN033883 | STm36 | Cold chicken | SP | 1995 | STR | — | — | — | — | — | — |
| CFSAN033884 | STm37 | Raw pork sausage | SP | 1996 | SUL | — | — | — | — | — | — |
| CFSAN033885 | STm38 | Human feces | SP | 1997 | SUL | — | — | — | — | — | — |
| CFSAN033886 | STm39 | Human feces | SP | 1998 | STR | — | — | — | — | — | — |
| CFSAN033887 | STm40 | Lettuce | SP | 1998 | SUL | — | — | — | — | — | — |
| CFSAN033888 | STm41 | Raw kafta | SP | 1998 | TET-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033889 | STm42 | Raw kafta | SP | 1998 | TET-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033890 | STm43 | Human feces | SP | 2000 | STR | — | — | — | — | — | — |
| CFSAN033891 | STm44 | Blood | SP | 2000 | SUL | — | — | — | — | — | — |
| CFSAN033892 | STm45 | Raw pork sausage | SP | 2000 | TET-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033893 | STm46 | Raw tuscan sausage | SP | 2000 | STR | — | — | — | — | — | — |
| CFSAN033894 | STm47 | Human feces | SP | 2003 | SUL | — | — | — | — | — | — |
| CFSAN033895 | STm48 | Brain abscess | SP | 2005 | AMP-SXT-STR-SUL | — | — | sul2 (100) | dfrA1 (100) | blaTMDaB (100) | — |
| CFSAN033896 | STm49 | Human feces | SP | 2010 | NA | — | — | — | — | — | — |
| CFSAN033897 | 702/99 | Final product | SC | 1999 | — | — | — | — | — | — | — |
| CFSAN033898 | 12280/06 | Swine | SC | 2006 | AMP-TET-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033899 | 12278/06 | Swine | SC | 2006 | NA-TET-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033900 | 12290/06 | Swine | SC | 2006 | TET-STR-SUL | aph(3")-Ib (100), aph(6)-Id (100) | sul2 (100) | — | — | — | — |
| CFSAN033901 | 12281/06 | Swine | SC | 2006 | AMP-NA-STR-SUL | strA (100), aph(6)-Id (100) | tetB (100) | — | — | — | — |
| CFSAN033902 | 12282/06 | Swine | SC | 2006 | TET-STR-SUL | aph(6)-Id (100), aph(3")-Ib (100) | sul2 (100) | — | — | — | — |
| CFSAN033903 | 5936/06 | Cold chicken | SC | 2006 | TET-SUL | — | — | — | — | — | — |
| CFSAN033904 | 5937/06 | Cold chicken | SC | 2006 | — | — | — | — | — | — | — |
| CFSAN033905 | 5938/06 | Swine | SC | 2006 | — | — | — | — | — | — | — |
| CFSAN033906 | 5939/06 | Swine | SC | 2006 | NA-TET-STR-SUL | strA (100), aph(6)-Id (100), aac(3)-Ia (99.87) | sul1 (99.89) | — | — | — | — |
| CFSAN033907 | 5940/06 | Swine | SC | 2006 | TET-STR-SUL | aadA1 (99.75), strA (100), aph(6)-Id (100) | sul1 (99.89) | — | — | — | — |
| CFSAN033908 | 5941/06 | Poultry | SC | 2006 | TET-SUL | — | — | — | — | — | — |
| CFSAN033909 | 13609/06 | Poultry | SC | 2006 | — | — | — | — | — | — | — |
| CFSAN033910 | 16202/09 | Ready-to-eat | RS | 2009 | TET-SUL | — | — | — | — | — | — |
| CFSAN033911 | 16203/09 | Ready-to-eat | RS | 2009 | TET-SUL | — | — | — | — | — | — |
| CFSAN033912 | 16204/09 | Ready-to-eat | RS | 2009 | TET-SUL | — | — | — | — | — | — |
| CFSAN033913 | 16205/09 | Industrialized | RS | 2009 | TET-SUL | — | — | — | — | — | — |
| CFSAN033914 | 16206/09 | Industrialized | RS | 2009 | TET-SUL | — | — | — | — | — | — |
| CFSAN033915 | 16207/09 | Industrialized | GO | 2009 | TET-SUL | — | — | — | — | — | — |

(Continued)
Table 1. (Continued)

| CFSAN n° | Isolate name | Source | State | Year of isolation | Phenotypic Resistance Profile | Genotypic Resistance Profile (Identity %) |
|----------|--------------|--------|-------|-------------------|-------------------------------|------------------------------------------|
| CFSAN033916 | 16273/09 | Industrialized product | GO | 2009 | AMP-NA-TET-GEN-SUL | tet(B) (100) |
| CFSAN033917 | 17307/09 | Industrialized product | - | 2009 | AMP-NA-SXT-TET-GEN-STR-SUL | tet(A) (100), sul2 (100), dfrA1 (100) |
| CFSAN033918 | 9481/10 | In natura meat | SC | 2010 | SUL | sul2 (100) |
| CFSAN033919 | 9479/10 | In natura meat | SC | 2010 | SUL | sul2 (100) |
| CFSAN033920 | 7022/10 | Poultry | PR | 2010 | CTX-AMF-AMP-SXT-TET-STR-SUL | sul2 (100) |
| CFSAN033921 | 3057/10 | Frozen chicken carcass | PR | 2010 | STR-SUL | sul2 (100) |
| CFSAN033922 | 6346/10 | Chicken | SP | 2010 | SUL | sul2 (100) |
| CFSAN033923 | 5633/10 | Unknown | RS | 2010 | NA | sul2 (100) |
| CFSAN033924 | 9399/10 | Swine | PR | 2010 | SUL | sul2 (100) |
| CFSAN033925 | 462/10 | Chicken | SC | 2010 | CTX-FEP-AMP-SUL-CIO | sul2 (100) |
| CFSAN033926 | 447/10 | Chicken | SC | 2010 | CTX-FEP-AMP-SUL-CIO | sul2 (100) |
| CFSAN033927 | 2452/11 | Frozen chicken carcass | SP | 2011 | TET-SUL | sul2 (100) |
| CFSAN033928 | 6799/11 | Cold chicken | RS | 2011 | AMP-NA-SXT-C-TET-GEN-SUL | sul2 (100) |
| CFSAN033929 | 948/12 | Raw salad | BA | 2012 | SUL | sul2 (100) |
| CFSAN033930 | 1103/12 | Raw salad (homemade salami) | RS | 2012 | SUL | sul2 (100) |
| CFSAN033931 | 1104/12 | Raw salad (homemade salami) | RS | 2012 | SUL | sul2 (100) |
| CFSAN033932 | 3300/12 | Roast beef | SC | 2012 | SUL | sul2 (100) |
| CFSAN033933 | 994/13 | Final product sales (animal origin) | SP | 2013 | SUL | sul2 (100) |
| CFSAN033934 | 374/13 | Final product sales (animal origin) | SC | 2013 | SUL | sul2 (100) |
| CFSAN033935 | 465/13 | Final product sales (animal origin) | SP | 2013 | AMP-SXT-TET-GEN-SUL | sul2 (100), dfrA1 (100) |
| CFSAN033936 | 622/13 | Final product sales (animal origin) | SC | 2013 | NA | sul2 (100) |
| CFSAN033937 | 583/13 | Final product sales (animal origin) | SC | 2013 | AMP-TET-SUL | sul2 (100), dfrA1 (100) |
| CFSAN033938 | 623/13 | Final product sales (animal origin) | SP | 2013 | AMP-NA-C-TET-STR | sul2 (100), dfrA1 (100) |

* This genome was the only one that presented the mph(A) (identity 100%) gene that confers resistance to macrolide.

https://doi.org/10.1371/journal.pone.0201882.t001
Institute (CLSI) guidelines. Strains with MIC \( \leq 0.06 \) \( \mu \)g/mL were considered sensitive and \( \geq 1 \) \( \mu \)g/mL resistant.

Phylogenetic analysis

In addition to the 90 S. Typhimurium strains sequenced in this study, four additional S. Typhimurium strains (the sequencing reads were downloaded from NCBI with run accessions of SRR1060710, SRR1963606, SRR6325339, and ERR1556230 for strain DT104, LT2, 14028s, and SL1344, respectively) were added into the phylogenetic analysis for diversity purpose. The genomic analysis was performed using the CFSAN SNP Pipeline that generated the SNP matrix, which was then used to infer the maximum likelihood tree using GARLI [34] with 200 maximum likelihood replicates and 1000 bootstrap iterations. Three samples were included as outgroups including: Salmonella enterica serovar Saintpaul CFSAN000611; Salmonella enterica serovar Saintpaul CFSAN000614; and Salmonella enterica serovar Heidelberg CFSAN000443 [35]. The SNP matrix included 59,130 and 11,176 SNPs, with or without the three outgroups sample, respectively.

Results

\textit{In silico} antimicrobial resistance gene analysis

A total of 39 antimicrobial resistance genes were identified (Table 1) and are described in detail below according to the different antimicrobial classes.

\textbf{Aminoglycoside resistance genes.} Ten distinct aminoglycoside resistance genes were detected including: the most common gene \texttt{aadA1} in 23 (25.6\%) isolates (19 humans, 4 foods); \texttt{aph(6)-Id} in 20 (22.2\%) isolates (7 humans, 13 foods); \texttt{aph(3')-Ia} in 11 (12.2\%) isolates (10 humans, 1 foods); \texttt{ant(2'')-Ia} in 7 (7.8\%) isolates from humans; \texttt{aacA4} in 5 (5.6\%) isolates from humans; and \texttt{aph(3'')-Ib} in 5 (5.6\%) isolates (1 humans, 4 foods); \texttt{aph(4)-Ia} in 3 (3.3\%) isolates from foods; \texttt{aac(3)-IVa} in 3 (3.3\%) isolates from foods; and lastly both \texttt{aac(3)-IIId} and \texttt{aadA15} in 1 (1.1\%) food isolate each.

\textbf{Tetracycline resistance genes.} Five distinct tetracycline resistance genes were detected including: the most common \texttt{tet(B)} gene in 19 (21.1\%) isolates (3 humans, 16 foods); \texttt{tet(A)} in 8 (8.9\%) food isolate; \texttt{tet(C)} in 7 (7.8\%) human isolates; \texttt{tet(M)} in 3 (3.3\%) food isolates; and \texttt{tet(D)} in 1 (1.1\%) food isolate.

\textbf{Sulfonamide and trimethoprim resistance.} Only two sulfonamide resistance genes were detected including: \texttt{sul1} in 19 (21.1\%) strains (12 humans 7 foods); and \texttt{sul2} in 9 (10\%) strains (2 humans 7 foods). The 4 trimethoprim resistance genes detected included: the most common \texttt{dfrA1} in 24 (26.7\%) isolates (22 human, 2 foods); \texttt{dfrA12} in 4 (4.4\%) isolates; \texttt{dfrA8} in 2 (2.2\%) foods; and \texttt{dfrA25} in 1 (1.1\%) food isolate.

\textbf{Beta-lactam resistance genes.} Seven distinct beta-lactam resistance genes were detected including: \texttt{bla\_TEM-1B} in 16 (17.8\%) strains (6 human,10 foods); \texttt{bla\_OXA-4} in 7 (7.8\%) human isolates; \texttt{bla\_OXA-17} in 5 (5.6\%) human isolates; \texttt{bla\_TEM-1A} in 2 (2.2\%) food isolates; and \texttt{bla\_CTX-M-8} in 2 (2.2\%) food isolates; \texttt{bla\_TEM-187} in 1 (1.1\%) human isolate; and \texttt{bla\_CTX-M-2} in 1 (1.1\%) food isolate.

\textbf{Fluoroquinolone resistance genes.} Five fluoroquinolone resistance genes were detected including: \texttt{aac(6')Ib-cr} in 5 (5.6\%) human isolates; \texttt{oqxA} in 4 (4.4\%) food isolates; \texttt{oqxB} in 4 (4.4\%) food isolates; and \texttt{qnrB2} and \texttt{qnrB88} each in one (1.1\%) food isolate.

\textbf{Phenicol resistance genes.} Two phenicol genes were detected including: \texttt{catA1} in 14 (15.6\%) human isolates; and \texttt{floR} in 5 (5.6\%) food isolates.

\textbf{Macrolide resistance genes.} Only one macrolide resistant gene (\texttt{mphA}) was detected in one food isolate.
Antimicrobial susceptibility testing

A total of 65 (72.2%) out of 90 S. Typhimurium strains studied were resistant to sulfonamides, 44 (48.9%) strains were streptomycin resistant, 27 (30%) strains were resistant to tetracycline, 21 (23.3%) strains were gentamicin resistant, and 7 (7.8%) strains were resistant to ceftriaxone. In our previously published paper (26), 34 strains were resistant to nalidixic acid (NalR). In this study we evaluated the reduced susceptibility to fluoroquinolones of 34 strains NalR and 12 strains susceptible to nalidixic acid (NalS). All the 12 NalS strains and 21 NalR strains studied were sensitive to ciprofloxacin (MIC ≤ 0.06 μg/ml), whereas 11 NalR strains presented intermediate resistance to this drug (MIC 0.12–0.5 μg/ml) and two NalR strains were resistant to ciprofloxacin. All the antimicrobial susceptibility test results were presented in Table 1.

Detection of mutations in the gyrA, gyrB, parC and parE genes and of the presence of qnr, qepA, oqxAB and aac(6’)-Ib-cr genes

A total of 33 (36.7%) out of 90 strains studied presented mutation points in the gyrA gene, with all being resistant to nalidixic acid (Table 2). The nonsynonymous points of mutation in the gyrA gene included: aspartate/glycine, Asp(87)→Gly in 21 strains; aspartate/asparagine, Asp (87)→Asn in 7 strains; serine/tyrosine, Ser(83)→Tyr in 4 strains; and serine/phenylalanine, Ser(83)→Phe in one strain. None of the strains had more than one mutation point (Table 2). One strain (5934/06 isolated from swine) NalR did not show mutation in the gyrA gene. Seven (7.8%) strains presented synonymous nucleotide mutation, and these strains were NalS (data not shown) suggesting undiscovered mutations. Thirty-two (35.6%) strains presented synonymous nucleotide mutation in the parC gene and 10 of those strains were NalR with, two strains resistant to ciprofloxacin (data not shown). No strains presented mutations in the parE gene.

The qnrB88 gene was found in 1 (1.1%) Brazilian strain that previously had been reported both in Klebsiella pneumoniae (GenBank: KX118608) and under another gene (qnrE1) found in Klebsiella pneumonia (GenBank: KY781949). Additionally, one strain had the qnrB2 gene present in Salmonella Bredeney (GenBank: FJ844401). The oqxAB gene was found in 4 (4.4%) strains. However, these genes diverged in having 6 mutations compared to the oqxAB of Salmonella Derby (GenBank: FN811184). The aac(6’)-Ib-cr gene was identified in 5 strains isolated from humans.

Phylogenetic analysis

The 90 S. Typhimurium strains studied were distributed into 2 major clades (designated A and B, Fig 1). Clade A comprised 34 (37.8%) strains with 7 isolated from humans between 1985 and 2010, and 27 isolated from food between 1998 and 2013. Thirty-four strains located in Clade A were isolated from South, Southeast and Midwestern Regions in Brazil. Of the 34 strains in Clade A, 15 strains (14 foods, 1 human) were resistant to three or more antimicrobial classes being multidrug-resistant (MDR). Clade B comprised 56 (62.2%) strains with 35 isolated from humans between 1983 and 2003, and 21 strains isolated from food between 1995 and 2013. Fifty-six strains located in Clade B were from South, Southeast, Northeast and Midwestern Regions in Brazil. Of the 56 strains in Clade B, 23 strains (18 humans, 5 foods) were MDR. All reference genomes added were grouped in clade B (DT104, SL1344, 14028s and LT2).

Discussion

In this study 90 S. Typhimurium strains isolated from food and humans in Brazil were sequenced by next generation sequencing technology to evaluate their antimicrobial resistance.
Table 2. Quinolone resistance profiles of the 90 *Salmonella* Typhimurium strains studied isolated from humans and food in various States between 1983 and 2013 in Brazil.

| CFSAN n° | Isolate Name | CIP E-test | QRDRs mutations |
|----------|--------------|------------|-----------------|
|          |              |            | *gyrA* mutation | *gyrB* mutation | *parC* mutation | *parE* mutation |
| CFSAN033848 | STm01        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033849 | STm02        | Intermediate | Asp(87) → Gly   |               |               |               |
| CFSAN033850 | STm03        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033851 | STm04        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033852 | STm05        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033853 | STm06        |            |                |               |               |               |
| CFSAN033854 | STm07        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033856 | STm09        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033857 | STm10        | Intermediate | Asp(87) → Gly   |               |               |               |
| CFSAN033858 | STm11        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033859 | STm12        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033860 | STm13        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033861 | STm14        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033862 | STm15        |            |                |               |               |               |
| CFSAN033863 | STm16        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033864 | STm17        |            |                |               |               |               |
| CFSAN033865 | STm18        |            |                |               |               |               |
| CFSAN033866 | STm19        |            |                | Asp(87) → Gly |               |               |
| CFSAN033867 | STm20        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033868 | STm21        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033869 | STm22        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033870 | STm23        |            |                |               |               |               |
| CFSAN033871 | STm24        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033872 | STm25        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033873 | STm26        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033874 | STm27        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033875 | STm28        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033876 | STm29        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033877 | STm30        |            |                |               |               |               |
| CFSAN033878 | STm31        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033879 | STm32        |            |                |               |               |               |
| CFSAN033880 | STm33        |            |                |               |               |               |
| CFSAN033881 | STm34        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033882 | STm35        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033883 | STm36        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033884 | STm37        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033885 | STm38        |            |                |               |               |               |
| CFSAN033886 | STm39        |            |                |               |               |               |
| CFSAN033887 | STm40        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033888 | STm41        |            |                |               |               |               |
| CFSAN033889 | STm42        |            |                |               |               |               |
| CFSAN033890 | STm43        |            |                |               |               |               |
| CFSAN033891 | STm44        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033892 | STm45        | Susceptible | Asp(87) → Gly   |               |               |               |
| CFSAN033893 | STm46        | Susceptible | Asp(87) → Gly   |               |               |               |

(Continued)
### Table 2. (Continued)

| CFSAN n°  | Isolate Name | CIP E-test | QRDRs mutations |
|-----------|--------------|------------|-----------------|
|           |              |            | *gyr*A mutation | *gyr*B mutation | *par*C mutation | *par*E mutation |
| CFSAN033894 | STm47 | Susceptible | – | – | – | – |
| CFSAN033895 | STm48 | – | – | – | – | – |
| CFSAN033896 | STm49 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033897 | 702/99 | – | – | – | – | – |
| CFSAN033898 | 12288/06 | – | – | – | – | – |
| CFSAN033899 | 12278/06 | Susceptible | Asp(87)→Asn | – | – | – |
| CFSAN033900 | 12290/06 | – | – | – | – | – |
| CFSAN033901 | 12268/06 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033902 | 12381/06 | – | – | – | – | – |
| CFSAN033903 | 5936/06 | – | – | – | – | – |
| CFSAN033904 | 5937/06 | – | – | – | – | – |
| CFSAN033905 | 5934/06 | Susceptible | – | – | – | – |
| CFSAN033906 | 5961/06 | – | – | – | – | – |
| CFSAN033907 | 5962/06 | – | – | – | – | – |
| CFSAN033908 | 5929/06 | – | – | – | – | – |
| CFSAN033909 | 13609/06 | – | – | – | – | – |
| CFSAN033910 | 3848/08 | – | – | – | – | – |
| CFSAN033911 | 16238/09 | Resistant | Ser(83)→Tyr | – | – | – |
| CFSAN033912 | 16239/09 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033913 | 16240/09 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033914 | 16202/09 | – | – | – | – | – |
| CFSAN033915 | 16251/09 | – | – | – | – | – |
| CFSAN033916 | 16273/09 | Intermediate | Ser(83)→Phe | – | – | – |
| CFSAN033917 | 17307/09 | Resistant | Ser(83)→Tyr | – | – | – |
| CFSAN033918 | 9461/10 | – | – | – | – | – |
| CFSAN033919 | 9479/10 | – | – | – | – | – |
| CFSAN033920 | 7032/10 | – | – | – | – | – |
| CFSAN033921 | 3057/10 | – | – | – | – | – |
| CFSAN033922 | 6346/10 | – | – | – | – | – |
| CFSAN033923 | 5635/10 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033924 | 9109/10 | – | – | – | – | – |
| CFSAN033925 | 426/10 | – | – | – | – | – |
| CFSAN033926 | 447/10 | – | – | – | – | – |
| CFSAN033927 | 2452/11 | – | – | – | – | – |
| CFSAN033928 | 6709/11 | Intermediate | Asp(87)→Asn | – | – | – |
| CFSAN033929 | 948/12 | – | – | – | – | – |
| CFSAN033930 | 1103/12 | – | – | – | – | – |
| CFSAN033931 | 1104/12 | – | – | – | – | – |
| CFSAN033932 | 3330/12 | – | – | – | – | – |
| CFSAN033933 | 994/13 | – | – | – | – | – |
| CFSAN033934 | 374/13 | – | – | – | – | – |
| CFSAN033935 | 465/13 | – | – | – | – | – |
| CFSAN033937 | 622/13 | Intermediate | Ser(83)→Tyr | – | – | – |
| CFSAN033938 | 583/13 | – | – | – | – | – |
| CFSAN033939 | 623/13 | Intermediate | Ser(83)→Tyr | – | – | – |

[https://doi.org/10.1371/journal.pone.0201882.t002](https://doi.org/10.1371/journal.pone.0201882.t002)
gene profiles and phylogenetic diversity. This is the first study of S. Typhimurium strains isolated in Brazil that used WGS to access the genetic diversity and the molecular bases of antimicrobial resistance. In previous studies, the same strains were typed by PFGE, MLVA, ERIC-PCR, CRISPR-MVLST and MLST [26–28].

In this study, 47 (52.2%) strains presented phenotypic resistance to gentamicin and/or streptomycin. Streptomycin is not frequently used to treat Salmonella enterica infections; but, it has been commonly used as a growth promoter in food-producing animals and for this reason may serve as a marker for resistant strains moving through the food supply [11].

Our results confirm McDermott et al.’s. [11] observations of discrepancies between phenotypic resistance and genotypic resistance of aminoglycoside resistant genes. We observed 35 isolates carrying streptomycin resistance genes, but these isolates were phenotypically susceptible to the drugs. It is unclear why the genes while present in the genomes were not expressed to provide phenotypic resistance. Presence of the known streptomycin resistance genes does not predict phenotypic resistance well for this class.

The tetracycline resistance genes were found in 32 (35.5%) strains. Interestingly, 2 strains that were phenotypically resistant to tetracycline did not present any known tetracycline resistance genes suggesting a possible alternative mode of resistance. In contrast, seven strains that presented tetracycline resistance genes were phenotypically susceptible. Of these seven, six strains had two tetracycline resistance genes and one strain had only one tetracycline resistance gene. Tetracycline has been used commonly as an antibiotic in swine husbandry [36]. Brazil is a major producer of pigs with 3.73 million tons of pork produced and exported in 2016 [37, 38]. The Salmonella Typhimurium serovar usually does not cause severe disease in pigs and sometimes it is asymptomatic in these animals, which may be a serious public health problem, since it may be an important source of contamination of carcasses in slaughterhouses. In addition, the contamination by S. Typhimurium may not be detected while the pigs are on the farm, which may eventually lead to human contamination [36, 39].

Cefoxitin resistance has been used to indicate certain types of beta-lactamases production by Salmonella and E. coli. First and second-generation cephalosporin susceptibility results are not reported in clinical medicine for Salmonella, because the drugs may appear active in vitro, but are not therapeutically effective [33]. Regarding the beta-lactam resistance genes found in Brazil, the most common was blatem-1b gene presented in 16 (17.8%) isolates (6 humans, 10 foods). The blatem-1b gene has been associated with ampicillin resistance and 32 (35.6%) strains were phenotypically resistant to the ampicillin. The blctx-M-8 and blctx-M-2 genes have been more closely associated to cephalosporin resistance and 7 strains were resistant to ceftriaxone (CRO), third generation cephalosporin, but only 3 strains presented a blctx allele. The most common resistant gene was aac(6')ib-cr found in 5 (5.6%) human isolates followed by oqxA and oqxB found in 4 (4.4%) food isolates. The qnrB2 and qnrB88 genes were found each in 1 (1.1%) food isolate.

Some of the discrepancies observed when a resistance gene is present but no phenotypic resistance in bacterial growth is observed, or when the phenotype is present but no known resistance gene is observed, is likely due to new unidentified resistance genes or mutations conferring resistance in undiscovered genes. Therefore, it is important to study any discrepancy as each represents new ways that bacteria are acquiring resistance as was reported for a new mechanisms discovered for Campylobacter gentamicin resistance [40]. Pribul et al. [41]
evaluated the prevalence of PMRQ genes in 129 isolates of non-typhoidal Salmonella from Brazil by PCR amplification. Qnr genes were found in 15 (11.6%) isolates (8 qnrS, 6 qnrB, and 1 qnrD), and the aac(6\')-Ib gene was found in 23 (17.8%) isolates. Regarding mutation points in the QRDRs, gyrA mutation was the only one found among the strains studied. Thirty-three (36.7%) of nalidixic acid resistant strains presented mutations in the gyrA gene (22 human, 11 foods).

McDermott and colleagues [11] used WGS technology to identify known antimicrobial resistance genes among 640 non-typhoidal Salmonella strains for 43 different serotypes and correlated these with susceptibility phenotypes to evaluate the utility of WGS for antimicrobial resistance surveillance. Overall, genotypic and phenotypic resistance correlated in 99.0% of the cases. They concluded that WGS is an effective tool for predicting antibiotic resistance in non-typhoidal Salmonella [11]. Regarding QRDR mutations and PMQR genes, 21 isolates had either QRDR mutations or PMQR genes, all of which were from human clinical cases. In contrast, in this study QRDR mutations were found in both human and food isolates.

Salmonella Typhimurium ST313 had been described only in sub-Saharan Africa, with high levels of antibiotic resistance associated with bloodstream infections and mortality rates of >25% [42, 43]. In 2017 [28], nine strains were typed as ST313 in Brazil, with only 1 MDR, human strain (STm29 feces), presenting resistant to ampicillin, streptomycin and sulfonamide. Five Brazilian strains (STm30, STm35, STm37, STm47, STm44) were resistant just to sulfonamide with STm37 isolated from food. Other resistant strains included: STm40 isolated from food (streptomycin and sulfonamide); STm39 isolated from human feces (streptomycin); and STm34 isolated from human feces (pan_resistant).

Food isolates were distributed in Clades A and B in relatively similar numbers suggesting that there is more than one subtype in circulation, in foods in Brazil. Human's isolates were more prevalent in the Clade B suggesting the existence of a prevalent subtype. Genomic and phenotypic testing results suggest clinical strains isolated before the mid-1990s presented more antimicrobial resistance compared to later strains. The diversity and prevalence of resistant genes found in Brazilian Salmonella Typhimurium is an alert of their potential hazard for food safety and public health.

Supporting information

S1 Table. Characteristics of the 90 Salmonella Typhimurium genomes studied. (XLSX)
2. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011; 17(1):7–15. https://doi.org/10.3201/eid1701.P111011 PMID: 21192848

3. Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AW, Wegener HC, et al. Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis. 2011; 8(8):887–900. https://doi.org/10.1089/fpd.2010.0787 PMID: 21492021

4. Taunay AE, Fernandes SA, Tavechio AT, Neves BC, Dias AM, Irino K. The role of public health laboratory in the problem of salmonellosis in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 1996; 38 (2):119–27. PMID: 9071031

5. Tavechio AT, Fernandes SA, Neves BC, Dias AM, Irino K. Changing patterns of Salmonella serovars: increase of Salmonella enteritidis in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 1996; 38(5):315–22. PMID: 9293072

6. Tavechio AT, Ghilardi AC, Peresi JT, Fuzihara TO, Yonamine EK, Jakabi M, et al. Salmonella serotypes isolated from nonhuman sources in São Paulo, Brazil, from 1996 through 2000. J Food Prot. 2002; 65 (6):1041–4. PMID: 12092719

7. Fernandes SA, Tavechio AT, Ghilardi AC, Dias AM, Almeida IA, Melo LC. Salmonella serovars isolated from humans in São Paulo State, Brazil, 1996–2003. Rev Inst Med Trop Sao Paulo. 2006; 48(4):179–84. PMID: 17119671

8. Allard MW, Luo Y, Strain E, Li C, Keys CE, Son I, et al. High resolution clustering of Salmonella enterica serovar Montevideo strains using a next-generation sequencing approach. BMC Genomics. 2012; 13:32. https://doi.org/10.1186/1471-2164-13-32 PMID: 22260654

9. Cao G, Meng J, Strain E, Stones R, Pettengill J, Zhao S, et al. Phylogenetics and differentiation of Salmonella Newport lineages by whole genome sequencing. PLoS One. 2013; 8(2):e55687. https://doi.org/10.1371/journal.pone.0055687 PMID: 23409020

10. Hoffmann M, Zhao S, Pettengill J, Luo Y, Monday SR, Abbott J, et al. Comparative genomic analysis and virulence differences in closely related salmonella enterica serotype heidelberg isolates from humans, retail meats, and animals. Genome Biol Evol. 2014; 6(5):1046–68. https://doi.org/10.1093/gbe/evu079 PMID: 24732280

11. McDermott PF, Tyson GH, Kabera C, Chen Y, Li C, Folster JP, et al. Whole-Genome Sequencing for Detecting Antimicrobial Resistance in Nontyphoidal Salmonella. Antimicrob Agents Chemother. 2016; 60(9):5515–20. https://doi.org/10.1128/AAC.01030-16 PMID: 27381390

12. Wang H, Wang J, Yu P, Ge P, Jiang Y, Xu R, et al. Mutations in Nalidixic Acid-Resistant Serovar Typhimurium in Brazil. J Mol Med. 2017; 39(2):364–72. https://doi.org/10.3892/ijmm.2016.2844 PMID: 28035408

13. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis. 2001; 32(2):263–8. https://doi.org/10.1086/318457 PMID: 11170916

14. Fábrega A, Vila J. Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev. 2013; 26(2):308–41. https://doi.org/10.1128/CMR.00066-12 PMID: 23534419

15. Angulo FJ, Johnson KR, Tauxe RV, Cohen ML. Origins and consequences of antimicrobial-resistant nontyphoidal salmonella: implications for the use of fluoroquinolones in food animals. Microb Drug Resist. 2000; 6(1):77–83. https://doi.org/10.1089/mdr.2000.6.77 PMID: 10868811

16. Choi SH, Woo JH, Lee JE, Park SJ, Choo EJ, Kwak YG, et al. Increasing incidence of quinolone resistance in human non-typhoidal Salmonella enterica isolates in Korea and mechanisms involved in quinolone resistance. J Antimicrob Chemother. 2005; 56(6):1111–4. https://doi.org/10.1093/jac/dki369 PMID: 16244086

17. Campioni F, Souza RA, Martins VV, Stehling EG, Bergamini AMM, Falcão JP. Prevalence of gyrA Mutations in Nalidixic Acid-Resistant Strains of Salmonella Enteritidis Isolated from Humans, Food, Chickens, and the Farm Environment in Brazil. Microb Drug Resist. 2017; 23(4):421–8. https://doi.org/10.1089/mdr.2016.0024 PMID: 27554419

18. Malbok K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant Salmonella enterica serotype typhimurium DT104. N Engl J Med. 1999; 341(19):1420–5. https://doi.org/10.1056/NEJM199911043411902 PMID: 10547404

19. Helms M, Vastrup P, Gerner-Smidt P, Malbok K. Excess mortality associated with antimicrobial drug-resistant Salmonella typhimurium. Emerg Infect Dis. 2002; 8(5):490–5. https://doi.org/10.3201/eid0805.010267 PMID: 11996684

20. Yoshida H, Bogaki M, Nakamura M, Yamanaka LM, Nakamura S. Quinolone resistance-determining region in the DNA gyrase gyrB gene of Escherichia coli. Antimicrob Agents Chemother. 1991; 35 (8):1647–50. PMID: 1656869
21. Saenz Y, Zarazaga M, Briñas L, Ruiz-Larrea F, Torres C. Mutations in gyrA and parC genes in nalidixic acid-resistant Escherichia coli strains from food products, humans and animals. J Antimicrob Chemother. 2003; 51(4):1001–5. https://doi.org/10.1093/jac/dkg168 PMID: 12654733

22. Hopkins KL, Day M, Threlfall EJ. Plasmid-mediated quinolone resistance in Salmonella enterica, United Kingdom. Emerg Infect Dis. 2008; 14(2):340–2. https://doi.org/10.3201/eid1402.070573 PMID: 18258138

23. Casas MR, Camargo CH, Soares FB, da Silveira WD, Fernandes SA. Presence of plasmid-mediated quinolone resistance determinants and mutations in gyrase and topoisomerase in Salmonella enterica isolates with resistance and reduced susceptibility to ciprofloxacin. Diagn Microbiol Infect Dis. 2016; 85(1):85–9. https://doi.org/10.1016/j.diagmicrobio.2016.01.016 PMID: 26971183

24. Cummings KJ, Rodriguez-Rivera LD, Norman KN, Ohta N, Scott HM. Identification of a Plasmid-Mediated Quinolone Resistance Gene in Salmonella Isolates from Texas Dairy Farm Environmental Samples. Zoonoses Public Health. 2017; 64(4):305–7. https://doi.org/10.1111/zph.12318 PMID: 27801549

25. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. Microbiol Spectr. 2014; 2(5).

26. Almeida F, Medeiros MI, Rodrigues DoP, Falcão JP. Genotypic diversity, pathogenic potential and the resistance profile of Salmonella Typhimurium strains isolated from humans and food from 1983 to 2013 in Brazil. J Med Microbiol. 2015; 64(11):1395–407. https://doi.org/10.1099/jmm.0.000158 PMID: 26307078

27. Almeida F, Medeiros MI, Rodrigues DD, Allard MW, Falcão JP. Molecular characterization of Salmonella Typhimurium isolated in Brazil by CRISPR-MVLST. J Microbiol Methods. 2017; 133:55–61. https://doi.org/10.1016/j.mimet.2016.12.020 PMID: 28034696

28. Almeida F, Seribelli AA, da Silva P, Medeiros MIC, Dos Prazeres Rodrigues D, Moreira CG, et al. Multilocus sequence typing of Salmonella Typhimurium reveals the presence of the highly invasive ST313 in Brazil. Infect Genet Evol. 2017; 51:41–4. https://doi.org/10.1016/j.meegid.2017.03.009 PMID: 28262827

29. Campioni F, Falcão JP. Genotypic diversity and virulence markers of Yersinia enterocolitica biotype 1A strains isolated from clinical and non-clinical origins. APMIS. 2014; 122(3):215–22. https://doi.org/10.1111/apm.12126 PMID: 23763723

30. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013; 20(10):714–37. https://doi.org/10.1089/cmb.2013.0084 PMID: 24093227

31. Klimke W, Agarwala R, Badretdin A, Chetverin S, Ciufio S, Fedorov B, et al. The National Center for Biotechnology Information’s Protein Clusters Database. Nucleic Acids Res. 2009; 37(Database issue): D216–23. https://doi.org/10.1093/nar/gkn734 PMID: 18940865

32. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012; 67(11):2640–4. https://doi.org/10.1093/jac/dks261 PMID: 22782487

33. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

34. Davis S, Pettengill JB, Luo Y, Payne J, Shpuntoff A, Rand H, et al. CFSAN SNP Pipeline: an automated method for constructing SNP matrices from next-generation sequence data Peer J Computer Science. 2015; 1(e20):1–11.

35. Timme RE, Pettengill JB, Allard MW, Strain E, Barrangou R, Wehnes C, et al. Phylogenetic diversity of the enteric pathogen Salmonella enterica subsp. enterica inferred from genome-wide reference-free SNP characters. Genome Biol Evol. 2013; 5(11):2109–23. https://doi.org/10.1093/gbe/evt159 PMID: 24158624

36. Almeida F, Medeiros MI, Kich JD, Falcão JP. Virulence-associated genes, antimicrobial resistance and molecular typing of Salmonella Typhimurium strains isolated from swine from 2000 to 2012 in Brazil. J Appl Microbiol. 2016; 120(6):1677–90. https://doi.org/10.1111/jam.13110 PMID: 26913928

37. Viott AM, Lage AP, Cruz EC, Guedes RM. The prevalence of swine enteropathogens in Brazilian grower and finish herds. Braz J Microbiol. 2013; 44(1):145–51. https://doi.org/10.1590/S1517-83822013005000033 PMID: 24159297

38. ABPA. Annual Report. Brazil: Brazilian Association of Animal Protein; 2017. p. 1–68.

39. Kich JD, Colebelha A, Morès N, Nogueira MG, Cardoso M, Fratamico PM, et al. Prevalence, distribution, and molecular characterization of Salmonella recovered from swine finishing herds and a slaughter facility in Santa Catarina, Brazil. Int J Food Microbiol. 2011; 151(3):307–13. https://doi.org/10.1016/j.ijfoodmicro.2011.09.024 PMID: 22024043
40. Zhao S, Mukherjee S, Chen Y, Li C, Young S, Warren M, et al. Novel gentamicin resistance genes in Campylobacter isolated from humans and retail meats in the USA. J Antimicrob Chemother. 2015; 70 (5):1314–21. https://doi.org/10.1093/jac/dkv001 PMID: 25645207

41. Pribul BR, Festivo ML, Rodrigues MS, Costa RG, Rodrigues EC, de Souza MM, et al. Characteristics of Quinolone Resistance in Salmonella spp. Isolates from the Food Chain in Brazil. Front Microbiol. 2017; 8:299. https://doi.org/10.3389/fmicb.2017.00299 PMID: 28352250

42. Feasey NA, Cain AK, Msefula CL, Pickard D, Alaerts M, Aslett M, et al. Drug resistance in Salmonella enterica ser. Typhimurium bloodstream infection, Malawi. Emerg Infect Dis. 2014; 20(11):1957–9. https://doi.org/10.3201/eid2014.141175 PMID: 25340988

43. Ley B, Le Hello S, Lunguya O, Lejon V, Muyembe JJ, Weill FX, et al. Invasive Salmonella enterica serotype typhimurium infections, Democratic Republic of the Congo, 2007–2011. Emerg Infect Dis. 2014; 20(4):701–4. https://doi.org/10.3201/eid2004.131488 PMID: 24655438