Insights about the Epidemiology of Salmonella Typhimurium Isolates from different Sources in Brazil using Comparative Genomics

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Research Article

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Abstract

Background: *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. Typhimurium*) is an important zoonotic agent worldwide. In Brazil, there are few published studies that have characterized the possible differences of *S. Typhimurium* strains isolated from humans, foods and animal by whole genome sequencing (WGS). The aim of this work was to compare genetically 117 *S. Typhimurium* isolates from different sources from 30 years (1983 to 2013) in Brazil using different genomics strategies, including: phylogenetic analysis; orthologous protein clusters analysis; Multi-locus sequence typing (MLST); prophages and resistance genes screening related to efflux pumps.

Results: The majority of the 117 *S. Typhimurium* strains studied were grouped into a single cluster ($\cong 90\%$) by the genome core (cgMLST) and ($\cong 77\%$) by single copy marker genes (ggTree). The different orthologous protein clusters found for some *S. Typhimurium* strains isolated from humans and food are involved in metabolic and regulatory processes. For 26 *S. Typhimurium* isolates from swine the sequence type (ST) 19 was the most common, the ST1921 was the second most prevalent and the STs 14, 64, 516 and 639 were also detected. The main prophages detected were: Gifsy-2 in 79 (67.5%) and Gifsy-1 in 63 (54%) *S. Typhimurium* isolates. All of the *S. Typhimurium* isolates contained the *acrA*, *acrB*, *macA*, *macB*, *mdtK*, *emrA*, *emrB*, *emrR* and *tolC* efflux pump genes.

Conclusions: Phylogenetic analyses grouped the majority of the *S. Typhimurium* isolates into a single cluster suggesting that there is one prevalent subtype that has successfully contaminated human, food and animal sources for 30 years in Brazil. The orthologous protein clusters analysis revealed unique genes in the *S. Typhimurium* studied mainly related to bacterial metabolism and that may be important in their pathogenicity. *S. Typhimurium* isolates from swine showed greater diversity of STs and prophages in comparison to *S. Typhimurium* strains isolated from humans and foods. The pathogenic potential of *S. Typhimurium* strains was corroborated by the presence of exclusive prophages of this serovar involved in their virulence. The high number of resistance genes related to efflux pumps is worrying and may lead to therapeutic failures when treatment is needed.

Background

Nontyphoidal *Salmonella* (NTS) strains have been an important enteric agent transmitted mainly by contaminated foods worldwide [1]. According to Kirk and collaborators [2], it was estimated that 153 million infections and 56,969 deaths occurred around the globe due to salmonellosis in 2010. Moreover, data from the Centers for Disease Control and Prevention (CDC), estimated that 1.35 million infections, 26,500 hospitalizations and 420 deaths occur in the United States every year due to *Salmonella* [3].

In Brazil, *Salmonella* has been the first or second most common foodborne pathogen isolated from outbreaks in recent years [4]. However, until now there are few published studies that have characterized

the possible differences between Brazilian *S. Typhimurium* strains isolated from human, food and animal sources by whole genome sequencing (WGS).

S. Typhimurium is one of the main *Salmonella* generalist serovar, which has been isolated from pork in Europe, Oceania, Asia and North America, from poultry in North America and Oceania, from beef in Africa, Latin America and Europe, and from seafood in Europe [5]. Therefore, this serovar has been transmitted from animals and humans in different parts of the world and is characterized as a zoonotic agent causing losses of million of dollars for the meat producing industry [1, 6].

According to the Centers for Disease Control and Prevention (CDC), *S. Typhimurium* can also infect domestic pets and recently was responsible for an outbreak linked to contact with small pet turtles that affected 35 people from nine states and generated 11 hospitalizations [7].

WGS has been more accessible in the last few years and is used for molecular characterization studies [8]. Furthermore, different phylogenetic strategies can be performed after sequencing, such as construction of phylogenetic trees based on the core genome (cgMLST) and from single copy marker genes, comparison and analysis of orthologous protein clusters (OrthoVenn) and verification of the sequence type (ST) through Multilocus sequence typing (MLST) [9–11]. In addition, it has been possible to characterize the different prophages that contribute to *Salmonella* pathogenicity including identification of genes known to have functions such as virulence, metabolism and signaling [12].

It is important to emphasize that the monitoring of resistant NTS strains has been of great importance due to its continued emergence worldwide [13, 14]. According to Jajere, multidrug resistant (MDR) *Salmonella* has been a serious public health problem because it may lead to treatment failure when the use of antimicrobial is necessary [14]. In the United States, it was estimated that 212,500 infections and 70 deaths occur due to drug resistant NTS every year [13].

It is known that hundreds of genes can confer resistance to antibiotics in NTS and some were previously described for the *S. Typhimurium* strains isolated from humans and different foods in Brazil including genes related to resistance to aminoglycosides, tetracyclines, sulfonamides, trimethoprim, beta lactams, fluoroquinolones, phenicol and macrolides [15]. However, antibiotic resistance is multifactorial and little is known about resistance genes related to efflux pump, which can be an important factor that confers resistance to some antibiotics, such as fluoroquinolones, beta lactams, macrolides and aminoglycosides [15, 16].

The aim of this work was to compare genetically *S. Typhimurium* isolates from humans, food and swines in Brazil during 30 years using different genomics strategies, such as phylogenetic trees, protein orthologous clusters analysis, MLST, prophages and resistance genes related to efflux pump.

Results

cgMLST

The cgMLST grouped the 120 *S. Typhimurium* genomes studied in two main groups designated A and B (Fig. 1). Cluster A comprised 12 genomes of ST19 isolated from humans. Cluster B comprised a total of 108 genomes comprising strains isolated from humans, different foods and swines of ST19, ST1649, ST3343, ST1921 and ST313 in the case of strains isolated from humans and food, besides ST19, ST639, ST14, ST516, ST64 and ST1921 concerning strains isolated from swines. All three references were allocated in Cluster B. The CFSAN033848 and CFSAN033855 genomes isolated from humans were genetically distinct and did not grouped closely to any cluster.

Phylogenetic tree (ggTree) and orthologous protein clusters analysis

The phylogenetic tree grouped the 120 *S. Typhimurium* genomes studied in three groups designated A, B and C with cluster A subdivided in A.1 and A.2, cluster B subdivided in B.1 and B.2 (Fig. 2). Cluster A.1 comprised 84 genomes of ST19, ST1649, ST14, ST516, ST639, ST64, ST313, ST3343 and ST1921 isolated from humans, diverse foods and swines and the reference genomes. Cluster A.2 comprised nine genomes of ST19 isolated from humans, food and swines. Cluster B.1 comprised 20 genomes of ST19 from food and swines. Cluster B.2 comprised four genomes of ST19 isolated from human and food. Cluster C comprised three genomes of ST19 isolated from food and swine.

The orthologous protein clusters analysis was performed for the genomes that were more related to LT2, 14028S and D23580 references (Fig. 2). The comparisons indicated the orthologous protein clusters presented in the genomes of the strains of this study and absent in the references. The different unique orthologous protein clusters found are involved in metabolic and regulatory processes showed in detail in Table 2.

Table 2

– Unique orthologous protein clusters in some selected *S. Typhimurium* strains in comparison reference genomes

Groups	Biological processes (protein orthologous clusters)
LT2 – STm07, STm12, STm16, STm19, STm20, STm21, STm22, STm24, STm25, STm26 and STm27 (Comparison 1)	Transposition (DNA-mediated), Transposition, Viral procapsid maturation, Virion attachment to host cell, Viral genome integration into host DNA, Trehalose transport, DNA replication, Viral capsid assembly, DNA binding, Histidine catabolic process to glutamate and formate
LT2 – STm01 and STm08 (Comparison 2)	Transposition (DNA-mediated), Transposition, Viral genome integration into host DNA, Virion attachment to host cell, DNA replication, Trehalose transport, DNA replication initiation, Viral procapsid maturation, Formate oxidation, DNA binding
LT2 – STm04, STm09, STm10, STm11, STm13 and STm14 (Comparison 3)	Transposition (DNA-mediated), Transposition, Viral genome integration into host DNA, Trehalose transport, Histidine catabolic process to glutamate and formate
LT2 – STm02, STm03 and STm05 (Comparison 4)	Transposition (DNA-mediated), Transposition, Viral procapsid maturation, Viral genome integration into host DNA, Trehalose transport, DNA replication, Response to mercury ion, Mercury ion transmembrane transporter activity, Formate oxidation, DNA restriction-modification system
14028S – 13609/06, 6346/10, 9109/10, 9479/10, 948/12, 1103/12 and 1104/12 (Comparison 5)	Transposition (DNA-mediated), Transposition, Formate oxidation, Trehalose transport, Cell adhesion, DNA binding
D23580 – STm29, STm30, STm34, STm35, STm37, STm39, STm40, STm44 and STm47 (Comparison 6)	Transposition (DNA-mediated), Transposition, Formate oxidation, Trehalose transport, Lyase activity, Viral tail assembly, Cell adhesion
D23580 – STm29, STm30, STm34, STm35, STm36, STm37, STm39, STm40, STm44, STm47 and 3057/10 (Comparison 7)	Transposition (DNA-mediated), Formate oxidation, Trehalose transport, Lyase activity, Cell adhesion, Metal ion binding

MLST

Of the 26 *S. Typhimurium* strains isolated from swine studied, 16 (61.5%) belonged to the ST19, three (11.5%) to the ST1921, two (7.6%) to the ST14, two (7.6%) to the ST64, one (3.8%) to the ST516, one (3.8%) to the ST639 and one isolate did not match any known ST type.

Prophages detection

The Gifsy-2 prophage was detected in 79 (67.5%) *S. Typhimurium* isolates, Gifsy-1 in 63 (54%), Salmon 118970_sal3 in 46 (39%) and Haemop - HP1 in 21 (18%). Two dozen other prophages were also detected in the genomes studied and are described in detail in Table 3.

Table 3
– Proportion of intact prophages detected in the 117 *Salmonella* Typhimurium studied in Brazil

Prophages	Humans (n = 43) (%)	Foods (n = 48) (%)	Swine (n = 26) (%)
Aeromo_phiO18P	Not detected	48/01 (2.1)	26/01 (3.8)
Burkho_BcepMu	Not detected	48/01 (2.1)	Not detected
Edward_GF_2	43/02 (4.6)	48/06 (12.5)	Not detected
Entero_186	43/01 (2.3)	48/01 (2.1)	26/01 (3.8)
Entero_BP_4795	43/01 (2.3)	Not detected	Not detected
Entero_fiAA91_ss	Not detected	Not detected	26/06 (23.1)
Entero_mEp235	Not detected	Not detected	26/01 (3.8)
Entero_N15	43/03 (7)	Not detected	Not detected
Entero_P22	43/03 (7)	Not detected	Not detected
Entero_Tyrion	43/01 (2.3)	Not detected	Not detected
Entero_UAB_Phi20	43/05 (11.6)	Not detected	Not detected
Escher_RCS47	Not detected	Not detected	26/02 (7.7)
Gifsy_1	43/10 (23)	48/34 (71)	26/19 (73)
Gifsy_2	43/37 (86)	48/33 (68.7)	26/09 (34.6)
Haemop_HP1	43/18 (41.9)	Not detected	26/03 (11.5)
Salmon_118970_sal3	43/25 (58.1)	48/15 (31.2)	26/06 (23.1)
Salmon_118970_sal4	43/01 (2.3)	Not detected	Not detected
Salmon_epsilon34	Not detected	Not detected	26/01 (3.8)
Salmon_Fels_1	Not detected	48/01 (2.1)	Not detected
Salmon_Fels_2	43/04 (9.3)	48/01 (2.1)	26/04 (15.4)
Salmon_RE_2010	43/07 (16.3)	48/02 (4.2)	26/01 (3.8)
Salmon_SEN34	Not detected	Not detected	26/01 (3.8)
Salmon_SP_004	43/01 (2.3)	48/04 (8.3)	26/01 (3.8)
Salmon_SPN1S	43/17 (39.5)	Not detected	Not detected
Salmon_SPN3UB	Not detected	Not detected	26/02 (7.7)
Salmon_SPN9CC	Not detected	48/03 (6.2)	Not detected

Prophages	Humans (n = 43) (%)	Foods (n = 48) (%)	Swine (n = 26) (%)
Salmon_SSU5	Not detected	48/02 (4.2)	26/01 (3.8)
Salmon_ST64T	43/01 (2.3)	48/02 (4.2)	Not detected
Shigel_Sfll	Not detected	48/01 (2.1)	26/01 (3.8)

Efflux pump

The *acrA*, *acrB*, *macA*, *macB*, *mdtK*, *emrA*, *emrB*, *emrR* and *tolC* genes were detected in 117 (100%) *S. Typhimurium* isolates. The *mdsA* and *mdsB* genes were detected in 91 (100%) *S. Typhimurium* isolates from humans and different foods, but in only 18 (69.2%) *S. Typhimurium* isolates from swines. The *mdfA* gene was detected in 26 (100%) isolates from swines, 39 (81.2%) isolates from food and 18 (42%) isolates from humans. Finally, the *cmIA1* gene was detected only in isolates from swine 05 (19.2%). The percentage of query cover and identity for all genes ranged between 72–100 and 87–100, respectively (Table 4).

Table 4

– Frequencies of resistance genes related to efflux pumps in the 117 *Salmonella* Typhimurium studied

Genes	Humans (n = 43) (Query cover %) (Identity %)	Food (n = 48) (Query cover %) (Identity %)	Swine (n = 26) (Query cover %) (Identity %)
<i>acrA</i>	43/43 (100) (91.69)	48/48 (100) (91.69)	26/26 (100) (91.69)
<i>acrB</i>	43/43 (100) (94.66)	48/48 (100) (94.66)	26/26 (100) (94.66)
<i>macA</i>	43/43 (100) (88.65–88.84)	48/48 (100) (88.65–88.84)	26/26 (100) (88.39–88.84)
<i>macB</i>	43/43 (100) (88.60–88.70)	48/48 (100) (88.70)	26/26 (100) (88.29–88.70)
<i>mdtK</i>	43/43 (100) (99.79)	48/48 (100) (99.79)	26/26 (100) (99.16–99.79)
<i>emrA</i>	43/43 (100) (99.92–100)	48/48 (100) (100)	26/26 (72–100) (98.40–100)
<i>emrB</i>	43/43 (100) (95.7)	48/48 (100) (95.7)	26/26 (99.80–100) (95.7)
<i>emrR</i>	43/43 (100) (93.14)	48/48 (100) (93.14)	26/26 (100) (93.14)
<i>tolC</i>	43/43 (99–100) (100)	48/48 (100) (100)	26/26 (100) (98.78–100)
<i>mdsA</i>	43/43 (100) (99.75–100)	48/48 (100) (100)	26/18 (100) (100)
<i>mdsB</i>	43/43 (100) (100)	48/48 (100) (100)	26/18 (100) (100)
<i>mdfA</i>	43/18 (100) (87.93)	48/39 (100) (87.93)	26/26 (100) (87.93)
<i>cmIA1</i>	Not detected	Not detected	26/05 (100) (99.76)

Discussion

In this study, 117 *S. Typhimurium* isolates from humans (n = 43), food (n = 48) and swines (n = 26) in Brazil were compared using genomic analyses, such as phylogenetic neighbor joining, orthologous protein clusters detection, MLST analysis, and blast identification of prophages and resistance genes related to efflux pump.

The constructed neighbor joining trees grouped the 117 *S. Typhimurium* isolates into two and three groups, respectively. The majority of the 117 *S. Typhimurium* strains studied were grouped in a single cluster ($\cong 90\%$) by the genome core (cgMLST) and ($\cong 77\%$) by single copy marker genes (ggTree) suggesting that there is one prevalent subtype that has been successful in contaminating human, food and animal sources for 30 years in Brazil (Fig. 1 and Fig. 2). It is important to mention that the present study provided additional information about *S. Typhimurium* strains isolated from humans, food and swines in Brazil because such strains have never been studied together.

According to Jensen, homologous genes can be divided into orthologous and paralogs [17]. Orthologous genes are originated from a common ancestor during speciation events and kept the same function, on the other hand, paralogs genes are originated from duplication events and do not kept the same function [18].

Therefore, the OrthoVenn2 is a web server capable to annotate and compare orthologous protein clusters from the whole genome among different species [18]. In the present study, *S. Typhimurium* genomes more related to LT2, 14028S and D23580 references were compared and had their unique protein orthologous clusters revealed (Fig. 2). All *S. Typhimurium* isolates used for LT2 – comparison 1, 2, 3 and 4 were composed by a subcluster containing only strains of ST19 isolated from humans in the São Paulo State before the 1990s. The *S. Typhimurium* isolates used for 14028S – comparison 5 was formed by a subcluster containing only strains of ST19 isolated from food in the Rio Grande do Sul, Santa Catarina and Bahia States between 2006–2012. The *S. Typhimurium* isolates used for D23580 – comparisons 6 and 7 were formed by a subcluster containing strains of ST313 and ST19 isolated from humans and food in the São Paulo and Paraná States between 1995–2010.

Moreover, the different orthologous protein clusters found are involved in metabolic and regulatory processes, such as transposition, DNA replication, cell adhesion, formate oxidation, trehalose transport, lyase activity and response to mercury ion. These results show that despite being of the same serovar there are unique orthologous protein clusters in the strains studied in comparison to the reference strains and that may be important for their pathogenicity and were kept in these *S. Typhimurium* strains during natural selection and adaptation (Table 2).

In this study, MLST was performed only for swine isolates, because the STs for humans and food isolates were previously described in [19]. Of the 26 *S. Typhimurium* strains isolated from swine studied, 16 (61.5%) belonged to the ST19, three (11.5%) to the ST1921, two (7.6%) to the ST14, two (7.6%) to the ST64, one (3.8%) to the ST516, one (3.8%) to the ST639 and one did not have its ST detected.

According to Almeida and collaborators, the ST19 was the most common ST found, the ST313 was the second most prevalent. The STs 1649, 3343 and 1921 were also detected among the *S. Typhimurium* strains isolated from humans and different foods in Brazil [19].

S. Typhimurium isolates from swine showed greater diversity in the seven housekeeping genes studied despite having a lower number of strains ($n = 26$) in comparison to the number of *S. Typhimurium* strains isolated from humans ($n = 43$) and food ($n = 48$). In strains from swine the ST19 was the most common, the ST1921 was the second most prevalent and the STs 14, 64, 516 and 639 were also detected.

Accessing the Enterobase for *Salmonella*, it was observed that 29,572 samples isolated from human, reptile, ovine, swine, poultry, food and bovine in France, Mexico, China, Germany, Scotland, Portugal, Qatar, Korea, Ireland, United States (US), United Kingdom (UK) and Denmark deposited belonged to the ST19. For the ST313 3,049 samples isolated predominantly from human in Kenya, Ethiopia, Zimbabwe, Malawi, Mali and Nigeria were deposited [10].

Moreover, the STs 1649, 3343 and 1921 were found for 16, 4 and 7 strains respectively, isolated from humans, livestock, food and swines in Venezuela, Ireland, US, UK, Colombia, Ecuador, Vietnam and Brazil [10]. Finally, the ST516, ST64, ST639 and ST14 were linked to 370, 3,850, 237 and 2,149 strains respectively, isolated from humans, poultry, food, aquatic animals, reptiles and the environment in the US, Mexico, Senegal, Germany, Portugal, Qatar, Canada, UK, India, Ghana, Thailand, Malaysia, Malta, Vietnam, Pakistan, Greece, France, Germany, China, Denmark, Scotland, Norway and South Korea [10].

In the present study, the Gifsy-2 prophage was detected in 79 (67.5%) *S. Typhimurium* isolates, Gifsy-1 in 63 (54%), Salmon 118970_sal3 in 46 (39%) and Haemop - HP1 in 21 (18%). Specifically, Gifsy-1 prophage was detected in 10 (23%) *S. Typhimurium* strains isolated from humans, 34 (71%) strains isolated from different foods and in 19 (73%) strains isolated from swine (Table 3). Gifsy-2 prophage was detected in 37 (86%) *S. Typhimurium* strains isolated from humans, 33 (68.7%) strains isolated from foods and 9 (34.6%) strains isolated from swine (Table 3).

It is important to be mentioned that Gifsy prophages carry genes that are related to virulence of *S. Typhimurium* in the host [20, 21]. The Gifsy-1 prophage encodes three genes involved in the intracellular survival of *Salmonella* spp. in the host, denominated *gogB* (leucine-rich repeat protein), *sarA* (anti-inflammatory response activator) and *pagK2*. In the same way, the Gifsy-2 prophage encodes a superoxide dismutase (*sodC1*) that contributes to the survival of *Salmonella* spp. destroying the toxic radicals of the host macrophages [12, 22]. It is important to emphasize that Gifsy prophages have been found only in *S. Typhimurium* strains, as well as the Fels-1 and Fels-2 prophages [21]. In the present study, Fels prophages were detected in four *S. Typhimurium* strains isolated from humans, two strains isolated from food and four strains isolated from swine (Table 3).

According to Brussow et al., the Fels-1 prophage encodes the *sodCIII* and *nanH* genes related to the production of superoxide dismutase and neuraminidase in *S. Typhimurium*, respectively [23].

Furthermore, the Fels-2 prophage carries genes that are apparently related to regulation and adhesion of *S. Typhimurium* to host cells [12].

The Gifsy and Fels prophages have already been described in *S. Typhimurium* isolated in various parts of the world, such as Australia, Europe, China, among others [24–26]. It is important to emphasize that other prophages were also found in the *S. Typhimurium* strains studied including Salmon 118970_sal3 and Haemop - HP1 (Table 3). Moreover, two dozen other prophages were detected in the *S. Typhimurium* strains studied, but there is less information about them related to pathogenicity and/or virulence of this serovar (Table 3).

In addition, *S. Typhimurium* isolates from swine showed 6 (23.1%) unique prophages despite having a lower number of strains analysed (n = 26) in comparison to *S. Typhimurium* strains isolated humans (n = 43) and food (n = 48) that presented 7 (16.3%) and 3 (6.25%) unique prophages, respectively, suggesting the greater diversity in these mobile genetic element for *S. Typhimurium* strains isolated from swine in Brazil (Table 3).

Resistance to multiple drugs in bacteria has been a serious public health problem worldwide [27]. It is known that there are four main mechanisms that can cause this resistance, such as target alteration, drug inactivation, decreased permeability and drug expulsion through the production of efflux pump [28].

In the present study, the *acrA*, *acrB*, *macA*, *macB*, *mdtK*, *emrA*, *emrB*, *emrR*, *tolC*, *mdsA*, *mdsB*, *mdfA* and *cmlA1* genes were detected among the *S. Typhimurium* strains isolated from humans, food and swines. All of the isolates contained the *acrA*, *acrB*, *macA*, *macB*, *mdtK*, *emrA*, *emrB*, *emrR* and *tolC* genes (Table 4). Other genes related to production efflux pump, such as *oqxAB* and *floR* were previously reported in [15].

The AcrAB efflux system has been described as responsible for the intrinsic resistance to many antibiotics that can be used in medical practice for the treatment of *S. Typhimurium*, such as fluoroquinolones and beta-lactams [16]. According to the World Health Organization (WHO), *Salmonella* spp. was described as a high priority category pathogen in fluoroquinolones resistance of the Global Priority Pathogens List [27].

The *macA* and *macB* genes encode proteins that characterize an efflux pump related to macrolides resistance [29, 30]. According to the Universal Protein Resource (UniProt), the *mdtK*, *emrA*, *emrB*, *emrR*, *mdsA*, *mdsB*, *mdfA* and *cmlA1* genes encode mainly proteins involved in multidrug efflux transporter and confers resistance to different antibiotics, such as aminoglycosides, tetracyclines, novobiocin, nalidixic acid, chloramphenicol and norfloxacin [30, 31]. Furthermore, the *tolC* gene has been described as important for the formation of some multidrug efflux systems (AcrAB, MacAB, EmrAB and MdsAB) in *S. Typhimurium* [31].

Conclusions

The phylogenetic trees grouped the majority of the *S. Typhimurium* isolates into a single cluster suggesting that there is one prevalent subtype that has been successful in contaminating human, food and animal sources for 30 years in Brazil. The orthologous protein clusters analysis revealed unique genes in the strains studied mainly related to bacterial metabolism that may be important in their pathogenicity. *S. Typhimurium* isolates from swine showed greater diversity of STs and prophages in comparison to *S. Typhimurium* strains isolated from humans and food. The pathogenic potential of *S. Typhimurium* strains was corroborated by the presence of exclusive prophages of this serovar involved in their virulence. The high number of resistance genes related to efflux pump is worrying and may cause therapeutic failures when treatment is needed. Altogether, this study provided relevant data on the genomic characterization of *S. Typhimurium* strains isolated from different sources in Brazil using WGS.

Methods

Bacterial strains

A total of 117 *S. Typhimurium* strains isolated from humans (43), food (48) and swines (26) between 1983 to 2013 in Brazil were studied (Table 1). These strains were selected from the collections of the Adolfo Lutz Institute of Ribeirão Preto (IAL-RP), of the Oswaldo Cruz Foundation from Rio de Janeiro (FIOCRUZ-RJ) and of the Brazilian Agricultural Research Corporation (EMBRAPA).

Whole genome sequencing

The whole genome sequencing of the 117 *S. Typhimurium* strains was performed on the NextSeq platform (Illumina) at the U.S. Food and Drug Administration (FDA), College Park, Maryland, USA. The genomes were assembled using the software SPAdes [32] and the quality of the assemblies were evaluated using the software QUAST [33] as described in [15].

cgMLST

The cgMLSTFinder 1.1 analysis from a set of reads was determined for all 117 *S. Typhimurium* genomes and three different references of this serovar were chosen, which included LT2, 14028S and D23580 using the services of the center for genomic epidemiology for *Salmonella* (Enterobase) available at

<https://cge.cbs.dtu.dk/services/cgMLSTFinder/> [10].

Phylogenetic tree (ggTree) and orthologous protein clusters analysis

Three different references of *S. Typhimurium* serovar were chosen, which included LT2 (GCF_000006945), 14028S (GCF_000022165) and D23580 (GCF_900538085), all with fully closed deposited genomes. To evaluate the evolutionary distance between the sequenced genomes and the three reference strains, a neighbor-joining tree was built with the ezTree algorithm [11] and ggTree R package [34, 35] (Fig. 2). Additional characterization of the orthologous protein clusters for some of the key *S. Typhimurium*

strains were performed. The phylogroups selected included: Comparison 1 - LT2 with 11 genomes (CFSAN033873, CFSAN033871, CFSAN033874, CFSAN033859, CFSAN033869, CFSAN033872, CFSAN033863, CFSAN033854, CFSAN033867, CFSAN033866 and CFSAN033868); Comparison 2 - LT2 with two genomes (CFSAN033855 and CFSAN033848); Comparison 3 - LT2 with six genomes (CFSAN033856, CFSAN033860, CFSAN033861, CFSAN033857, CFSAN033858 and CFSAN033851); Comparison 4 - LT2 with three genomes (CFSAN033852, CFSAN033849 and CFSAN033850); Comparison 5–14028S with seven genomes (CFSAN033930, CFSAN033931, CFSAN033919, CFSAN033924, CFSAN033922, CFSAN033929 and CFSAN033909); Comparison 6 - D23580 with nine genomes (CFSAN033882, CFSAN033877, CFSAN033887, CFSAN033886, CFSAN033876, CFSAN033881, CFSAN033894, CFSAN033891 and CFSAN033884); Comparison 7 - D23580 with 11 genomes (CFSAN033882, CFSAN033877, CFSAN033887, CFSAN033886, CFSAN033876, CFSAN033881, CFSAN033894, CFSAN033891, CFSAN033884, CFSAN033921 and CFSAN033883) via OrthoVenn2 [18] in order to determine unique features and metabolic pathways defining each group.

Multi-locus sequence typing

MLST was performed for all the 26 isolates *S. Typhimurium* from swine using the MLST 2.0 of the Center for Genomic Epidemiology for *Salmonella enterica* available in <https://cge.cbs.dtu.dk/services/MLST/> [36]. The STs of the *S. Typhimurium* isolates from humans and different foods were previously described in [19].

Prophages detection

The genomes of all 117 *S. Typhimurium* strains were used to search the prophages by PHAge Search Tool Enhanced Release (PHASTER) that is an online platform for the rapid identification and annotation of prophages sequences in bacterial genomes and plasmids available in <http://phaster.ca/> [37].

Efflux pump

The genomes of all 117 *S. Typhimurium* strains were used to search resistance genes related to efflux pump. Resistance gene identifier (RGI) is part of the Comprehensive Antibiotic Resistance Database (CARD) and was performed with high quality/coverage (includes contigs > 20,000 bp and excludes prediction of partial genes) available in <https://card.mcmaster.ca/analyze/rgi> [38].

Abbreviations

NTS: Nontyphoidal *Salmonella*

CDC: Centers for Disease Control and Prevention

WGS: Whole genome sequencing

cgMLST: Core genome multilocus sequence typing

ST: Sequence type

MLST: Multilocus sequence typing

MDR: Multidrug resistant

DNA: Deoxyribonucleic acid

WHO: World Health Organization

UniProt: Universal Protein Resource

FDA: Food and Drug Administration

PHASTER: PHAge Search Tool Enhanced Release

RGI: Resistance gene identifier

CARD: Comprehensive Antibiotic Resistance Database

IAL-RP: Adolfo Lutz Institute of Ribeirão Preto

FIOCRUZ-RJ: Oswaldo Cruz Foundation from Rio de Janeiro

EMBRAPA: Brazilian Agricultural Research Corporation

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data from 117 *S. Typhimurium* genomes were deposited in the GenBank (NCBI) under the identification numbers: LVHC000000000, LVHB000000000, LVHA000000000, LVGZ000000000, LVGY000000000, LVGX000000000, LVGW000000000, MABI000000000, LVGV000000000, LVGU000000000, LVGT000000000, LUJG000000000, LVGS000000000, LVGR000000000, LVGQ000000000, LVGP000000000, LVGO000000000, LVGN000000000, LVGM000000000, LVGL000000000, LUJF000000000, LVGK000000000, LVGJ000000000, LVGI000000000, LVGH000000000, LVGG000000000, LVGF000000000, LUJE000000000, LVGE000000000, LVGD000000000, LUJD000000000, LVGC000000000, LVGB000000000, LVGA000000000,

LVFZ00000000, LVFY00000000, LVFX00000000, LUJC00000000, LUJB00000000, LUJA00000000, LVFW00000000, LUIZ00000000, LVFV00000000, LVFU00000000, LUIY00000000, LVFT00000000, LUIX00000000, LUIW00000000, LVFS00000000, LVFR00000000, LUIV00000000, LUIU00000000, LUIT00000000, LVFQ00000000, LUIS00000000, LUIR00000000, LUIQ00000000, LUIP00000000, LUIO00000000, LVFP00000000, LUIN00000000, LUIM00000000, LUIL00000000, LUIK00000000, LVFO00000000, LUJJ00000000, LUII00000000, LUIH00000000, LVFN00000000, LUIG00000000, LUIF00000000, LUIE00000000, LVFM00000000, LUID00000000, LUIC00000000, LVFL00000000, LVFK00000000, LUJB00000000, LUJA00000000, LUHZ00000000, LVFJ00000000, LUHY00000000, LUHX00000000, LVFI00000000, LUHW00000000, LUHV00000000, LUHU00000000, LUHT00000000, LUHS00000000, LUHR00000000, LVFH00000000, SRR8291813, [SRR8291805](#), [SRR8291802](#), [SRR8291817](#), [SRR8291806](#), [SRR8291814](#), PHJE00000000, PHJD00000000, PHJC00000000, PIJC00000000, PHJB00000000, PHJA00000000, PHIZ00000000, PHIY00000000, PHIX00000000, PHIW00000000, PHIV00000000, PHIU00000000, PHIT00000000, PHIS00000000, PHIR00000000, PHIQ00000000, PHIP00000000, PHIO00000000, PHIN00000000, PHIM00000000, released under the project PRJNA186035 (<https://www.ncbi.nlm.nih.gov/bioproject/186035>).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AAS participated in the design of the work, performed to methods, analysed and interpreted all the data and wrote the original manuscript. PS performed to methods. MFC analysed and interpreted the data. FA performed the whole genome sequencing of the *S. Typhimurium* strains isolated from humans and foods. MRF performed the whole genome sequencing of the *S. Typhimurium* strains isolated from swine. MICM collected *S. Typhimurium* strains isolated from humans and foods. DPR collected *S. Typhimurium* strains isolated from foods. JDK collected *S. Typhimurium* strains isolated from swine. LJB performed to methods. SCS performed to methods. MWA was whole genome sequencing supervisor. JPF participated in the design of the work and supervisor. All authors read and approved the final manuscript.

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Table

Table 1
Characteristics of the 117 *Salmonella* Typhimurium strains studied isolated from different sources in Brazil

CFSAN nº	Isolate name	Source	State	Year of isolation	Sequence Type (ST)
CFSAN033848	STm01	Human feces	SP	1983	19
CFSAN033849	STm02	Human feces	SP	1983	19
CFSAN033850	STm03	Human feces	SP	1983	19
CFSAN033851	STm04	Human feces	SP	1983	19
CFSAN033852	STm05	Human feces	SP	1983	19
CFSAN033853	STm06	Human feces	SP	1983	1649
CFSAN033854	STm07	Human feces	SP	1983	19
CFSAN033855	STm08	Human feces	SP	1983	19
CFSAN033856	STm09	Human feces	SP	1984	19
CFSAN033857	STm10	Human feces	SP	1984	19
CFSAN033858	STm11	Human feces	SP	1984	19
CFSAN033859	STm12	Human feces	SP	1984	19
CFSAN033860	STm13	Human feces	SP	1984	19
CFSAN033861	STm14	Human feces	SP	1984	19
CFSAN033862	STm15	Human feces	SP	1985	3343
CFSAN033863	STm16	Human feces	SP	1985	19
CFSAN033864	STm17	Human feces	SP	1985	19
CFSAN033865	STm18	Human feces	SP	1985	19
CFSAN033866	STm19	Human feces	SP	1986	19
CFSAN033867	STm20	Human feces	SP	1986	19
CFSAN033868	STm21	Human feces	SP	1986	19
CFSAN033869	STm22	Human feces	SP	1986	19
CFSAN033870	STm23	Human feces	SP	1986	19
CFSAN033871	STm24	Human feces	SP	1986	19
CFSAN033872	STm25	Human feces	SP	1986	19
CFSAN033873	STm26	Human feces	SP	1986	19

CFSAN nº	Isolate name	Source	State	Year of isolation	Sequence Type (ST)
CFSAN033874	STm27	Human feces	SP	1986	19
CFSAN033875	STm28	Human feces	SP	1988	3343
CFSAN033876	STm29	Human feces	SP	1989	313
CFSAN033877	STm30	Human feces	SP	1990	313
CFSAN033878	STm31	Human feces	SP	1991	19
CFSAN033879	STm32	Human feces	SP	1992	19
CFSAN033880	STm33	Human feces	SP	1992	19
CFSAN033881	STm34	Human feces	SP	1993	313
CFSAN033882	STm35	Human feces	SP	1995	313
CFSAN033883	STm36	Cold chicken	SP	1995	19
CFSAN033884	STm37	Raw pork sausage	SP	1996	313
CFSAN033885	STm38	Human feces	SP	1997	19
CFSAN033886	STm39	Human feces	SP	1998	313
CFSAN033887	STm40	Lettuce	SP	1998	313
CFSAN033888	STm41	Raw kafta	SP	1998	19
CFSAN033889	STm42	Human feces	SP	1999	19
CFSAN033890	STm43	Human feces	SP	2000	19
CFSAN033891	STm44	Blood	SP	2000	313
CFSAN033892	STm45	Raw pork sausage	SP	2000	19
CFSAN033893	STm46	Raw tuscan sausage	SP	2002	19
CFSAN033894	STm47	Human feces	SP	2003	313
CFSAN033895	STm48	Brain abscess	SP	2005	19
CFSAN033896	STm49	Human feces	SP	2010	19
CFSAN033897	702/99	Final product	SC	1999	19
CFSAN033898	12288/06	Swine	SC	2006	19
CFSAN033899	12278/06	Swine	SC	2006	19
CFSAN033900	12290/06	Swine	SC	2006	19

CFSAN nº	Isolate name	Source	State	Year of isolation	Sequence Type (ST)
CFSAN033901	12268/06	Swine	SC	2006	19
CFSAN033902	12381/06	Swine	SC	2006	19
CFSAN033903	5936/06	Cold chicken	SC	2006	19
CFSAN033904	5937/06	Cold chicken	SC	2006	19
CFSAN033905	5934/06	Swine	SC	2006	19
CFSAN033906	5961/06	Swine	SC	2006	19
CFSAN033907	5962/06	Swine	SC	2006	19
CFSAN033908	5929/06	Poultry	SC	2006	19
CFSAN033909	13609/06	Poultry	SC	2006	19
CFSAN033910	3848/08	Food	SC	2008	19
CFSAN033911	16238/09	Ready-to-eat dish	MS	2009	19
CFSAN033912	16239/09	Ready-to-eat dish	MS	2009	19
CFSAN033913	16240/09	Ready-to-eat dish	MS	2009	19
CFSAN033914	16202/09	Industrialized product	RS	2009	19
CFSAN033915	16251/09	Industrialized product	GO	2009	19
CFSAN033916	16273/09	Industrialized product	GO	2009	19
CFSAN033917	17307/09	Industrialized product	-	2009	19
CFSAN033918	9461/10	In natura meat	SC	2010	19
CFSAN033919	9479/10	In natura meat	SC	2010	19
CFSAN033920	7032/10	Poultry	PR	2010	19
CFSAN033921	3057/10	Frozen chicken carcass	PR	2010	19
CFSAN033922	6346/10	Chicken	SP	2010	19
CFSAN033923	5635/10	Unknown	RS	2010	19
CFSAN033924	9109/10	Swine	PR	2010	19
CFSAN033925	426/10	Chicken	SC	2010	19
CFSAN033926	447/10	Chicken	SC	2010	19
CFSAN033927	2452/11	Frozen chicken carcass	SP	2011	19

CFSAN nº	Isolate name	Source	State	Year of isolation	Sequence Type (ST)
CFSAN033928	6709/11	Cold chicken	RS	2011	19
CFSAN033929	948/12	Raw salad	BA	2012	19
CFSAN033930	1103/12	Swine (homemade salami)	RS	2012	19
CFSAN033931	1104/12	Swine (homemade salami)	RS	2012	19
CFSAN033932	3330/12	Roast beef	SC	2012	19
CFSAN033933	994/13	Final product sales (animal origin)	SP	2013	19
CFSAN033934	374/13	Final product sales (animal origin)	SC	2013	19
CFSAN033935	465/13	Final product sales (animal origin)	SP	2013	19
CFSAN033937	622/13	Final product sales (animal origin)	SC	2013	1921
CFSAN033938	583/13	Final product sales (animal origin)	SC	2013	19
CFSAN033939	623/13	Final product sales (animal origin)	SC	2013	1921
CFSAN068033	739	Mesenteric lymph node	SC	2000	ST19
CFSAN034668	1030	Swine feces	SC	2003	ST19
CFSAN068028	22	Inguinal lymph node	SC	2004	ST516
CFSAN034669	29	Swine feces	SC	2004	ST639
CFSAN034670	51	Swab swine carcass	SC	2004	ST14
CFSAN034671	68	Swine feces	SC	2004	ST14
CFSAN068029	58	Swine faeces	SC	2004	ST19
CFSAN068040	1212	Mesenteric lymph node	SC	2005	ST19
CFSAN068043	1218	Mesenteric lymph node	SC	2005	ST19
CFSAN068044	1220	Mesenteric lymph node	SC	2005	ST19
CFSAN068045	1221	Mesenteric lymph node	SC	2005	ST19
CFSAN068046	1222	Mesenteric lymph node	SC	2005	ST19
CFSAN068047	1224	Mesenteric lymph node	SC	2005	ST19

CFSAN nº	Isolate name	Source	State	Year of isolation	Sequence Type (ST)
CFSAN068031	343	Herd environment	SC	2006	ST19
CFSAN068042	1214	Herd environment	SC	2006	ST19
CFSAN068041	1213	Herd environment	SC	2007	ST19
CFSAN068030	338	Swine faeces	SC	2008	ST19
CFSAN068032	345	Swine faeces	SC	2008	ST19
CFSAN068034	812	Swine urine	SC	2011	ST1921
CFSAN068035	824	Swine urine	SC	2011	ST1921
CFSAN068036	1206	Swab drag	SC	2011	ST19
CFSAN034672	804	Swine urine	SC	2011	ST1921
CFSAN034673	1209	Swab feeder	SC	2011	Unknown
CFSAN068037	1207	Swab carcass	SC	2012	ST19
CFSAN068038	1210	Swab carcass	SC	2012	ST64
CFSAN068039	1211	Swab carcass	SC	2012	ST64

Figures

Figure 1

Phylogenetic analysis with cgMLST profiles based on soft core of 3,002 genes selected for 117 *Salmonella* Typhimurium genomes isolated from humans (n=43), different foods (n=48) and swines (n=26) in Brazil.

Figure 2

Phylogenetic analysis based on a list of single copy marker genes for 117 *Salmonella* Typhimurium genomes isolated from humans (n=43), different foods (n=48) and swines (n=26) in Brazil.