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ALINE PAROLIN CALARGA

Caracterização Fenotípica e Molecular de Amostras de *Salmonella enterica* Isoladas de Humanos no Estado de São Paulo

Molecular and Phenotypical Characterization of *Salmonella enterica* Isolated from Humans in São Paulo State

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Dissertation presented to the Biology Institute of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Genetics and Molecular Biology, in the area of Genetics of Microorganisms.

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Supervisor: Prof. Dr. Marcelo Brocchi

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COMISSÃO EXAMINADORA

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Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

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RESUMO

Salmonella enterica é um importante patógeno de humanos e outros animais que causa a zoonose denominada Salmonelose. Embora a maior parte dos casos de infecção humana seja causado pela *Salmonella enterica* subespécie *enterica,* o aparecimento de subespécies não-*enterica* invasivas têm sido relatado e associados ao contato com animais contaminados, principalmente anfíbios e répteis. Adicionalmente, o aumento do número de cepas multirresistentes de *Salmonella* spp. com maior patogenicidade tem sido descrito globalmente. Portanto, o presente trabalho teve por objetivo caracterizar o perfil fenotípico de susceptibilidade a antimicrobianos de amostras de *Salmonella* spp. isoladas de infecções humanas no estado de São Paulo entre 2000 e 2019, além de caracterizar o genótipo de resistência e virulência de isolados multirresistentes (MDR), Typhi e subespécie não-*enterica* através do sequenciamento completo do genoma. Dentre as 810 amostras estudadas, 43.82% foram resistentes a pelo menos um antibiótico. A maior parte das amostras apresentou resistência às seguintes classes de antimicrobianos: aminoglicosídeos (32.10%), tetraciclinas (13.81%) e β-lactâmicos (13.21%). Ademais, 71 isolados apresentaram perfil de multirresistência, 11 foram produtores de ESBL e um foi considerado resistente à colistina. De acordo com os dados do sequenciamento genômico todos os isolados sequenciados apresentaram bombas de efluxo relacionadas à resistência antimicrobiana. Dentre os quatro isolados MDR, três apresentaram genes AMR adicionais que estão associados à elementos móveis, como *blatem-1B, dfrA1/14, tetA, sul1/2, floR,* e *qnrE1*. Dentro do limite de nosso conhecimento o presente trabalho é o primeiro a descrever a presença do gene de resistência à quinolona mediado por plasmídeo (PMQR) *qnrE1* em um isolado clínico de *Salmonella* I 4,[5],12:i:-. Todos os isolados sequenciados tanto da subespécie *enterica* quanto não-*enterica* apresentaram divergências em relação a presença de "*Salmonella Pathogenicity Islands"* (SPIs) e bacteriófagos. Os bacteriófagos Gifsy-1 e 2 abrigaram importantes fatores de virulência como o gene *gogB* no isolado de variante monofásica, e o gene *sodC1* nos isolados de Enteritidis e de variante monofásica. Apesar das diferenças apresentadas, todos os isolados apresentaram importantes genes relacionados à invasão e sobrevivência intracelular das SPI-1/2/3. Adicionalmente, as amostras da subespécie não-*enterica* apresentaram fatores de virulência associados a outros patógenos como *Yersinia* spp. e *Escherichia coli* patogênicas. Apesar das deleções encontradas nas cinco principais SPIs e da ausência de SPI-6, entre as amostras da subespécie não-*enterica,* elas mostraram a habilidade de invadir e sobreviver macrófagos J774 assim como a cepa *Salmonella* Typhimurium ATCC 14028. Além do mais,

durante os ensaios *in-vivo* em *Galleria mellonella* os isolados não-*enterica* apresentaram taxas de mortalidades superiores à *S.* Typhimurium UK-1. Em conclusão, as amostras estudadas no presente trabalho apresentaram menor susceptibilidade ao aminoglicosídeos, tetraciclinas e β-lactâmicos, com uma baixa proporção de isolados apresentando perfil MDR, ou de produção de ESBL. Todavia, como apontado pelos dados de WGS, os isolados de MDR carregavam genes AMR em elementos móveis, o que pode contribuir para uma dispersão rápida destes genes entre amostras bacterianas. Em adição, mesmo que subespécies não-*enterica* sejam mais comuns entre animais de sangue frio, nosso estudo descreve pela primeira vez no Brasil isolados invasivos das subespécies *salamae* e *diarizonae* em infecções humanas.

Palavras-chave: *Salmonella enterica*; Resistência; Patogenicidade; Genômica

ABSTRACT

Salmonella enterica is an important pathogen to humans and other animals causing the zoonosis denominated as Salmonellosis. Although most cases of human-infections are caused by *Salmonella* subspecies *enterica*, the emergence of invasive non-*enterica* subspecies have been reported and associated to contact with infected animals, especially reptiles and amphibians. Additionally, the emergence of multidrug resistant strains (MDR) of *Salmonella* spp. with increased pathogenicity has been described worldwide. Therefore, this project aimed to characterize the antimicrobial resistance phenotype of *S. enterica* samples isolated from human infections in São Paulo state over the period of 2000 to 2019, besides characterizing the resistance and virulence genotype of MDR, Typhi and non-*enterica* subspecies samples through whole-genome sequencing (WGS). Among the 810 samples studied, 43.82% were resistant to at least one antibiotic. The majority of samples were resistant to the following antimicrobial classes: aminoglycosides (32.10%), tetracycline (13.83%) and β-lactams (13.21%). Moreover, 71 isolates were considered MDR, 11 were ESBL-producers and one was colistin-resistant. According to the whole-genome sequencing results all samples investigated presented efflux pumps correlated with antimicrobial resistance (AMR). Among the four MDR isolates sequenced, three presented additional AMR genes associated with mobile elements, such as $bla_{tem\text{-}1B}$, *dfrA1/14, tetA, sul1/2, floR,* and *qnrE1*. To the best of our knowledge the present work is the first to describe the plasmid-mediated quinolone resistance (PMQR) gene *qnrE1* in a clinical isolate of *Salmonella* I 4,[5],12:i:-. All sequenced samples subspecies *enterica* and non-*enterica* presented divergences among the presence of *Salmonella* Pathogenicity Islands (SPIs) and bacteriophages. The phages Gifsy-1/2 harbored important virulence factors likewise *gogB* in the monophasic variant isolate and *sodC1* in the monophasic variante and Enteritidis isolate. Despite divergences found, all samples harbored SPI-1/2/3 genes considered important to invasion and intracellular surveillance. In addition to that, the subspecies non-*enterica* samples presented virulence factors associated with other pathogens such as *Yersinia* spp. and pathogenic *Escherichia coli*. Despite deletions in SPI-1/3/4/5, and absence of SPI-6 found among the subspecies non-*enterica* samples, they have shown the ability to invade and survive within J774 macrophages likewise *S.* Typhimurium ATCC 14028. Furthermore, during the *in-vivo* assays with *Galleria mellonella* the subspecies non-*enterica* isolates presented higher killing rates compared

to *S.* Typhimurium UK-1. In conclusion, our work shows that a high percentage of samples herein tested showed a decreased susceptibility to aminoglycosides, teracyclines and β-lactams, with a small proportion of them presenting a MDR profile or ESBL-production. Nevertheless, as shown by WGS the MDR strains carried AMR genes presented in mobile elements, which can contribute to their rapid dissemination among bacterial strains. Moreover, although non-*enterica* subspecies are more prevalent among cold-blooded animals, our study describes for the first time in Brazil invasive isolates of subspecies *salamae* and *diarizonae* causing human-infections.

Keywords: *Salmonella enterica*; Resistance; Pathogenicity; Genomics

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LIST OF ABBREVIATIONS

µg – micrograms

µl – microliters

ACSSuT – Resistance- Type: ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline

AML – Amoxicillin

ANVISA – Agência Nacional de Vigilância Sanitária

ATCC – American Type Culture Collection

BLAST – Basic Local Alignment Search Tool

C – Chloramphenicol

C63PI – Centisome 63 Pathogenicity Island

CARD – Comprehensive Antibiotic Resistance Database

CATs – chloramphenicol acetyl-transferases

CAZ – Ceftazidime

CFU – Colony-forming unit

CIP – Ciprofloxacin

CLA – Clavulanic-acid

CLO – Cloxacillin

CLSI – Clinical and Laboratory Standard Institute

CN – Gentamicin

CT – Colistin

CTX – Cefotaxime

DI_{50} – Infective Dose

DNA - Deoxyribonucleic acid

EDTA - Ethylenediaminetetraacetic acid

ENR – Enrofloxacin

ESBL – Extended-Spectrum Beta-Lactamase

ETP – Ertapenem

FOX – Cefoxitin

GI – Genomic Islands

GP – Growth Promoters

h – Hours

H_2S – Sulfuric Acid

HPI – High-Pathogenicity Island

HU-USP – Hospital Universitário da Universidade Estadual de São Paulo

IAL – Instituto Adolfo Lutz

IL – Interleukin

iNTS – invasive non-typhoidal *Salmonella*

IPM – Imipenem

KCN – Potassium Cyanide Broth

KPC – Carbapenemase-producers

LB – Luria-Bertani medium

LEE – Locus of Enterocyte Effacement

LPEC – Lung Pathogenic *Escherichia coli*

MBC – Minimum Bactericidal Concentration

MDR - multi-drug resistance

MEM – Meropenem

mg – milligrams

MH – Mueller Hinton

MIC – Minimum Inhibitory Concentration

ml – milliliters

MLST – Multi-locus Sequence Type

mm – millimeters

mM – milliMolar

NCBI – National Center for Biotechnology Information

NCTC – National Collection of Type Cultures

nm – nanometers

NMEC – Neonatal meningintis-causing *Escherichia coli*

NS – Non-susceptible

NTS – non-Typhoidal *Salmonella*

ºC – Celsius degrees

ONPG – Ortho-nitrophenyl-β-D- galactopyranoside

PBS – Phosphate- Buffered Saline

pMLST – Plasmid Multi-locus Sequence Type

PMQR – Plasmid-Mediated Quinolone Resistance

pSEV – *Salmonella* Enteritidis Virulence Plasmid

pSV - *Salmonella* Virulence Plasmid

QRDR – Quinolone resistance-determining regions

rpm – revolutions per minute

RPMI - Roswell Park Memorial Institute Medium

RT-PCR – Real-Time Polymerase Chain Reaction

S – Streptomycin

S – Susceptible

SCV – *Salmonella*-containing vacuoles

SEF – *Salmonella* Enteritidis Fimbriae

Ser. – serovar

SGI – *Salmonella* Genomic Island

SNPs – Single Nucleotide Polymorphisms

SPI – *Salmonella* Pathogenicity Island

SXT – Sulfamethoxazole/ Trimethoprim

T3SS – Type III Secretion System

TE - Tetracycline

TLR – Toll-like Receptor

UPEC – Uropathogenic *Escherichia coli*

VFDB – Virulence Factors Database

WGS – Whole-Genome Sequencing

WHO – World Health Organization

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1. Introduction

Salmonella spp. is a gram-negative rod from the *Enterobacteriaceae* family and is the causative agent of Salmonellosis, the second most prevalent zoonose worldwide after *Campylobacter* spp. The transmission of this pathogen occurs mainly through contaminated food and water with most cases being linked to poultry products (Lamas et al., 2018). The genus *Salmonella* spp. is composed of two species denominated *enterica* and *bongori*. The species *enterica* is divided into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae,* and *indica.* Moreover, the species *bongori* as well as all *enterica* subspecies are classified into serotypes according to the composition of their somatic (O), flagellar (H) and capsular (Vi) antigens. The diversity of this genus allows it to affect a wide range of hosts such as mammals, reptiles, amphibians, fish, birds, and plants (Miranda et al., 2013). Furthermore, Salmonellosis can present different signs and symptoms such as asymptomatic patients, gastroenteritis, or acute systemic infections that will depend on the serotype of the infectious pathogen and host's condition (Hensel, 2004).

Salmonella enterica subspecies *enterica* is involved in most cases of human Salmonellosis and because of that, it is the most studied and reported among *Salmonella* subspecies. The *Salmonella enterica* serovars Typhi and Paratyphi present great epidemiological importance for causing enteric fever, an acute infection that affects only humans. Enteric fever presents higher morbidity and mortality in developing countries, especially among children below five years old and immunocompromised patients. In 2000 the World Health Organization estimated 33 million cases of typhoid fever around the world, with 600,000 fatal cases (Woc-Colburn e Bobak, 2009). Some regions in Africa and Asia are the most affected ones, with certain locations surpassing 100 cases per 100,000 individuals. However, the lack of consistency of surveillance systems, and data gaps around the world make it harder to understand the real number of cases and fatalities around the globe, which jeopardizes the implementation of programs to combat this pathogen (Buckle et al., 2012).

In addition to serotypes Typhi and Paratyphi, other serotypes denominated non-typhoidal *Salmonella* (NTS) from the same subspecies *enterica* have as well epidemiological importance due to their high prevalence in human-infections besides affecting food-producing animals. The most prevalent NTS serotypes are Enteritidis and Typhimurium causing gastrointestinal and extraintestinal infections in different continents (Hendriksen *et al.*, 2011).

In Africa, non-typhoidal serotypes affect 3.4 million people, while in Brazil around 39% of the foodborne diseases are correlated with NTS (MacFadden *et al.*, 2016; Ritter e Tondo, 2014). In meta-analysis study recently published 535000 cases of NTS infection worldwide were estimated with most cases being linkd to low-income countries and children below five years old (Stanaway et al., 2019). However, likewise, typhoidal *Salmonella* NTS infections are underreported and studies to evaluate this pathogen's incidence globally need to be taken.

Although most human-Salmonellosis is caused by subspecies *enterica,* other subspecies have been reported in human-infections around the world at lower rates. Studies have shown that non-*enterica* subspecies are part of reptiles and amphibians microbiota, thereby these animals are asymptomatic carriers of this pathogen being a source of infection. Although there are not enough studies about non-*enterica* virulence factors and infection routes, many cases of human-Salmonellosis were linked to owners of exotic pets, with both animals and owners carrying the same serotype (Mermin et al., 2004; Ward, 2000; Gay et al., 2014). Children that have contact with contaminated animals or with asymptomatic adults can develop meningitis, gastroenteritis, or sepsis and there were reports of fatal cases in infants infected with *S. diarizonae* (Giner-Lamia et al., 2019). Therefore, more studies need to be conducted about invasive non-*enterica* subspecies to understand their prevalence in symptomatic and asymptomatic patients and mitigate possible outbreaks.

Salmonellosis can be treated with antibiotics, but with the emergence of multidrug-resistant strains, the treatment is becoming more challenging. For instance, multi-drug resistance strains (MDR) of *S.* Typhi have been isolated since the end of 1980s showing resistance against ampicillin, chloramphenicol, and trimethoprim/ sulfamethoxazole, and this MDR pattern is still isolated in current cases of typhoid fever (Feasey *et al.*, 2015; Tatavarthy *et al.*, 2014; Wain *et al.*, 1999). Not only typhoidal-*Salmonella* has shown an MDR profile, but also NTS such as the resistance type ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline) found mostly among Typhimurium strains and is mediated by a transferable chromosomal element denominated *Salmonella* Genomic Island 1 (SGI-1) (Kiss *et al.*, 2012; Mulvey *et al.*, 2006). Furthermore, a great cause of concern regarding typhoidal and non-typhoidal *Salmonella* is the increasing number of Extended-Spectrum β-lactamases (ESBLs) strains that are resistant against broad-spectrum β-lactams (Crump et al., 2015). The production of these enzymes is associated with genes located in mobile elements; thereby this phenotype can be transferred among different serotypes, species, and bacterial genus (Crump et al., 2015; Ahmed *et al.*, 2012).

On the other hand, non-*enterica* subspecies isolated from human cases have shown to be susceptible to antimicrobials tested (Gerlach et al., 2017; Gay et al., 2014; Hervas et al., 2012; Bertrand et al., 2008). Even among non-human isolates of non-*enterica* subspecies have shown low rates of resistance. However, *Salmonella arizonae* and *Salmonella salamae* serovar Sofia isolated from food-producing animals showed resistance against antimicrobials commonly used in veterinary medicine, which highlights the importance of controlling measures to antimicrobials use to avoid selection and dissemination of resistance genes (Evangelopoulou et al., 2014; Lamas et al., 2018).

Therefore, *Salmonella* spp. presents great epidemiological importance due to its high prevalence among food and consequently humans. Considering the importance of studies that investigate the antimicrobial resistance and virulence characteristics of this pathogen in Brazil, our study aimed to characterize 810 samples of *Salmonella* spp. isolated from humans in São Paulo. First of all, the susceptibility profile of these samples was evaluated against 15 different antimicrobials through phenotype tests. Afterward, we have selected MDR, Typhi, and non-*enterica* subspecies samples to be further characterized by whole-genome sequencing to investigate their antimicrobial resistance genes, mobile elements, and virulence factors. Moreover, considering non-*enterica* subspecies have never been reported in human-infections in Brazil, we also performed *in-vivo* and *in-vitro* assays with these samples to compare their virulence phenotype with *Salmonella* spp. reference strains.

2. Aims of the study

2.1. General Aims

This study aims to characterize the phenotype and genetic features of *S. enterica* samples isolated from human infections in São Paulo state.

2.2. Specific Aims

- Characterize the antimicrobial resistance profile of the *S. enterica* samples using phenotypic tests;

- Further investigation of the antimicrobial resistance genes, virulence factors, and mobile elements presented by multi-drug resistant isolates, Typhi isolates, and non-*enterica* subspecies isolates.

- Characterize the virulence of *Salmonella* subspecies *diarizonae* and subspecies *salamae* isolates through macrophage invasion and survival assays, and *Galleria mellonella in-vivo* assays.

3. Literature Review

3.1. Classification and nomenclature of *Salmonella* spp.

The genus *Salmonella* belongs to the *Enterobacteriaceae* family and it is a gram-negative rod, facultative anaerobe, and most of the cases motile with peritrichous flagella (Crump *et al.*, 2015). This genus was named after the veterinarian Daniel E. Salmon, who isolated this pathogen for the first time during an outbreak of swine fever in 1885 (Grimont et al., 2007). The nomenclature and taxonomy of the genus *Salmonella* were first classified as one species *enterica* which was divided into six subgenera that were referred to by roman numerals (Table 01) (Brenner et al., 2000). After advances in molecular biology associated with biochemical characterization of *Salmonella* spp. the nomenclature was updated and currently the genus is divided into two species: *enterica* and *bongori.* The species *enterica* is now composed of six subspecies that were previously classified as subgenera (*enterica, salamae, arizonae, diarizonae, houtenae,* and *indica*), and the roman numerals are still used to group all subspecies and the species *bongori* as described by Table 01 (Miranda et al., 2013).

Moreover, the White (1926) and Kauffmann (1978) scheme divides the genus *Salmonella* into serotypes (serovars) which are classified based on the serologic identification of somatic (O), flagellar (H: phases 1 and 2), and capsular (Vi) antigens. Each of the serovars defined presents a unique antigenic formula which is the combination of different O-antigens and H-antigens resulting in more than 2600 different serotypes described to this date (Table 01) (Issenhuth-Jeanjean et al., 2014). The genetic Vi-antigen is only found among serovars Typhi and Paratyphi. Serovars from the subspecies *enterica* are usually referred to by names instead of their antigenic formula, and most of them were named after the geographical location where they were first isolated (*Salmonella enterica* serovar Minnesota), although some are named after their associated disease (*Salmonella enterica* ser. Typhi). When first cited the serotypes must be anticipated by the genus and species name in italic and then the serovar can be referred without the necessity of italic for not being a taxonomic classification, but subsequently, the complete name can be abbreviated to the genus name followed by the serovar or just the serovar's name (Brenner et al., 2000).

Table 01. Number of serovars according to the species and subspecies of *Salmonella* spp.

Species	**Subspecies/Group**	**Nº of serovars**
S. enterica	*enterica*/I	1586
	salamae/II	522
	arizonae/IIIa	102
	diarizonae/IIIb	338
	houtenae/IV	76
	indica/V	13
S. bongori	-/V	22
	Total	2659

Source: Miranda et al., 2013/ Issenhuth-Jeanjean et al., 2014

The biochemical characteristics of *Salmonella* spp. allow the differentiation from other *Enterobacteriaceae* family bacteria besides being possible to distinguish *Salmonella* subspecies. Moreover, in some cases serotypes within the same subspecies can present different biochemical characteristics complementing their serological classification. For instance, the *S. enterica* ser. Gallinarum is not motile, while *S. enterica* ser. Paratyphi does not produce H_2S. Table 02 presents some of the phenotypic tests that can be performed in order to differentiate *Salmonella* spp. strains based on their metabolism.

Table 02. Biochemical characteristics from each *Salmonella* subspecies and species.

Species	***enterica***						***bongori*** **(V)**
Subspecies /Traits	*enterica* (I)	*salamae* (II)	*arizonae* (IIIa)	*diarizonae* (IIIb)	*houtenae* (IV)	*indica* (VI)	-
Dulcitol	+	+	-	-	-	d	+
Lactose	-	-	d	d	-	d	-
ONPG	-	-	+	+	-	d	+
Salicin	-	-	-	-	+	-	-
Sorbitol	+	+	+	+	+	-	+
Malonate	-	+	+	+	-	-	-
Mucate	+	+	+	d	-	+	+
Gelatin	-	+	+	+	+	+	-
KCN	-	-	-	-	+	-	+
Indol	-	-	-	-	-	-	-
Motility	d	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-
H_2S	d	+	+	+	+	+	+

+: more than 90% of strains positive; -: more than 90% of strains negative; d: differences among each serotype; ONPG: ortho-nitrophenyl-β-D-galactopyranoside.; H_2S: Sulfuric Gas; KCN: Potassium Cyanide Broth. Adapted from: Grimont et al., (2000).

3.2.Pathogenicity

Salmonella enterica is the causative agent of Salmonellosis which is the second most prevalent foodborne infection worldwide, with the first being caused by *Campylobacter* spp. (Lamas et al., 2018). This bacterial infection can present different outcomes since asymptomatic patients or mild gastrointestinal infections to systemic infections, likewise typhoid fever (Hensel, 2004). Salmonellosis can be transmitted by the ingestion of contaminated water, vegetables, or meat, mostly poultry or swine derived products, but it can also be transmitted through direct or indirect contact with contaminated animals, such as reptiles or amphibians which are asymptomatic carriers of this pathogen (Lamas et al., 2018; Schikora et al., 2012). The human infective dose (DI_{50}) of Salmonella spp. is approximately 10^5-10^{10} organisms. This infective dose varies depending on the serotype, patient's age, immune system condition, and other characteristics (Kothary & Babu, 2001).

S. enterica has the ability to resist the acid pH found in the stomach as well as resisting to bile salts in the intestine. This process is mediated by physiological alterations of the bacteria such as the expression of stress response proteins, surface proteins, and active secretion systems (Spector and Kanyon, 2012). Moreover, this bacteria is a facultative intracellular pathogen, and once ingested it presents the ability to adhere and invading cells from the intestinal mucosa, preferentially M cells (Garai *et al.*, 2012). The fimbriae genes are involved with the initial adhesion to the host's cells mediating the colonization of the intestinal tract and participating in the invasion process (Wiedemann *et al.*, 2015). *S. enterica* expresses a wide variety of fimbriae genes or adhesins that diverge according to the serotype. For instance, the Lpf fimbriae *(long polar fimbriae*) play an important role in the Peyer's patch adhesion (Bäumler *et al.*, 1996).

Once adhered to the host's cells surface, the bacterial cells will invade epithelial cells, mostly M cells, through their disruption which results in bacteria's engulfment. However, other invasion mechanisms have been described, such as the translocation of bacterial cells by phagocytes and the disruption of the tight junctions by the bacteria (Haraga et al., 2008). Moreover, the invasion of non-phagocytic cells was previously believed to be mediated by only one type III secretion system (T3SS) encoded by SPI-1 (*Salmonella* pathogenicity islands) genes. Nevertheless, recent data indicated different invasion mechanisms, such as the zipper-like internalization mediated by protein Rck, or the invasion process mediated by PagN, besides other processes not completely elucidated (Boumart *et al.*, 2014).

After surpassing the intestinal mucosa barrier *S. enterica* invades, persists, and proliferates inside vacuoles of epithelial cells and reticuloendothelial system, which make possible the spreading of bacterial cells into different organs and tissues from the host resulting in systemic infection (Haraga et al., 2008).

S. enterica infections can result in acute inflammation of the intestinal barrier. This inflammation process is mostly correlated with the presence of the proteins that constitute the bacterium flagella called flagellins. Toll-like receptor 5 (TLR 5) can recognize flagellins in the extracellular environment (Gewirtz *et al.*, 2001), whereas in the intracellular environment those proteins are detected by the host via caspase 1 activation and IL-1β production within infected macrophages (Franchi *et al.*, 2006). Interestingly, the inflammation process triggered by *S. enterica* does not jeopardize its infection because these bacteria have acquired mechanisms to escape from the immune system cells, besides using the inflammation process in its own favor. For instance, *S. enterica* uses metabolites produced during the inflammation process, such as tetrathionate, as its final electron acceptor of the respiratory chain, and consequentely it presents an adaptative advantage when compared to the host's microbiota giving it (Rivera-Chávez e Bäumler, 2015).

Most of *S. enterica* virulence factors are grouped into Pathogenicity Islands (PIs) that are called *Salmonella* Pathogenicity Islands (SPIs), and they are currently divided into 23 SPIs (Hurley et al., 2014). Despite the high number of SPIs, not all serovars present all 23 intact SPIs, their presence varies among isolates. SPI-1 through SPI-5 have been described in a variety of serovars, but SPI-1 and SPI-2 are known for being essential in *Salmonella* spp. virulence being more conserved among *Salmonella* spp. serovars (Hurley et al., 2014). SPI-1, the first SPI to be described and the best characterized, in conjunction with SPI-2 are responsible for encoding the type III secretion system (T3SS) which is involved in rearranging the host's cytoskeleton and invading host's cells, as well as inducing inflammation in the intestinal mucosa (LaRock *et al.*, 2015).

The SPI-1 has been described as essential to invade non-phagocytic cells, to activate an inflammatory response, and to colonize the intestine. The T3SS genes encoded by this island are responsible for forming needle-like structures that play an important role in the translocation of virulence effectors into host cells allowing the bacteria to invade intestine's cells (Haraga et al., 2008). For instance, SptP, SipABC, and SopE are some of the effectors encoded in this island which functions are summarized in Table 03. The SptP and SipA

proteins disrupt epithelial cells' cytoskeleton helping the colonization process, but studies have shown that the *sptP* gene is not required for *in-vitro* invasion. SipB and SipC play an important role as apoptosis inducers besides being translocators of other SPI-1 factors. SopE activates factors that will also contribute to cytoskeleton rearrangement and inducing the inflammatory process (Haraga et al., 2008). Interestingly, mutant strains lacking one of these factors could still develop an effective invasion, showing evidence these genes might be redundant (Haraga et al., 2008). Another important virulence factor encoded by SPI-1 is the *invA* gene that plays an important role in the intestinal cells invasion process, but also seems to be involved in bacterial replication after the invasion step (Marcus et al., 2000; Haraga et al., 2008).

Another SPI that encodes T3SS proteins is the SPI-2. The T3SS genes presented in this island can be divided into four main groups of operons: *ssa* (T3SS apparatus), *ssr* (T3SS regulators), *ssc* (T3SS chaperones) and *sse* (T3SS effectors) (Table 03). In addition to these operons, SPI-2 T3SS also harbors *Salmonella* induced filaments (Sifs), these membrane structures play an important role in increasing the SCV size which allows the bacterial cells to replicate in high rates (Table 03). Mutant strains lacking SPI-2 have shown to lose the ability to replicate within the intracellular environment and spreading to other tissues, although they are still able to colonize intestinal cells (Marcus et al., 2000). Moreover, there is evidence showing SPI-2 and SPI-1 do not work independently, there is a cross-talk between these two islands, even though studies on the evolution of virulence genes in *Salmonella* spp. have shown these two SPIs were acquired independently in different times (Haraga et al., 2008).

The regulation of SPI-1 and SPI-2 is complex and not completely understood, but it is known that PhoP/Q, a two-component system, is responsible for modulating the SPI-2 gene expression. Furthermore, PhoP/Q also regulates the operon *mgtCB* in SPI-3 essential for macrophage survival (Haraga et al., 2008; Hurley et al., 2014).

Besides the SPI-1 and SPI-2 functions, the SPI-4, although not completely understood, is known for playing an important role in epithelial cells adhesion, while SPI-5 encodes *pipB* and *sopB* which are important virulence genes secreted and translocated by T3SS in SPI-1 and SPI-2 being necessary for an effective invasion and intracellular survival (Knodler et al., 2015; Hurley et al., 2014).

Table 03. Examples of virulence factors encoded by SPI-1 through SPI-5 in *Salmonella* spp.

Location	Gene	Function

SPI-1	*invA*	Delivery of T3SS effector proteins and
	sptP	Actin rearrangements
	sipA	Actin rearrangements
	sipB	Macrophages apoptosis and translocation of T3SS
	sipC	Translocation of T3SS
	sopE	Actin rearrangements and cytokine production
SPI-2	*sifAB*	Induces Sif formation and maintains integrity of SCV
	ssa group	T3SS apparatus
	ssaB	Interferes with endosomal trafficking
	sse group	T3SS effectors
	sseFG	Contributes to Sif formation
	sseI	Contributes to host-cell dissemination
	sseJ	Maintains integrity of the SCV
	ssr group	T3SS regulators
	ssc group	T3SS chaperones
SPI-3	*mgtBC*	Mg^{2+} uptake
SPI-5	*sopB*	Activates Cdc42, RhoG, AktA and chloride secretion through its inositol phosphatase activity and disrupts tight junctions
	pipB	Promote bacterial survival in host tissues

Adapted from: Marcus et al. (2000) and Haraga et al. (2008).

Once *Salmonella* spp. invades the host cells, it remains inside structures denominated SCV (*Salmonella-containing vacuole*), which are modified phagosomes where this pathogen will be able to survive and replicate. Morerover, recent data demonstrated that *S. enterica* can also manage to survive outside SCVs and it replicates within the cytoplasmic environment of non-phagocytic cells, consequently triggering an inflammatory response. During this stage, *S. enterica* can express flagellar genes and SPI-1. The eukaryotic cell ends up detaching from the epithelia, subsequently suffering apoptosis, which releases bacterial cells that will initiate the invasion process again (Behnsen *et al.*, 2015; Knodler, 2015; Boumart *et al.*, 2014).

As previously mentioned the presence of SPIs varies among serovars and subspecies and through the evolution process of this pathogen these gene clusters have been acquired,

and the only conserved SPI among both *Salmonella* species is the SPI-1 (Lamas et al., 2018). Regarding the species *enterica,* there are variations of SPIs presence among subspecies and this diversity allows us to understand their phylogenetic relationship. For instance, in previous work, the acquisition of SPI-18 marked the divergence of subspecies *arizonae* and *diarizonae* from the other subspecies. Moreover, the subspecies *salamae* and *indica* diverged from subspecies *enterica* later on and *salamae* divergence is marked by the gain of the locus of enterocyte effacement (LEE) as well as the loss of SPI-5 that harbors genes such as *pipB* and *sopB*. The subspecies *enterica* branching was correlated with the gain of SPI-6, an island associated with bacterial competition, and other virulence factors (Lamas et al., 2018). Nevertheless, there are little studies about the pathogenicity of non-*enterica* subspecies, and little genomics data available about them. Therefore, the few studies described in the literature are made based on a low number of strains and more studies are necessary to understand the diversity of virulence factors and mechanisms among subspecies. To date, studies have shown that non-*enterica* subspecies present several deletions in SPIs, especially among T3SS genes, correlated with invasion and surveillance resulting in lower pathogenicity. There are studies demonstrating evidence of an intracellular lifestyle of *diarizonae, salamae,* and *arizonae* strains that show their ability to adhere and invade eukaryotic cells. However, they poorly colonized and replicated within host cells, and most cases of human-infections are correlated with children and immunocompromised patients which might indicate these subspecies are opportunistic pathogens (Lamas et al., 2018).

The regulation of *S. enterica* pathogenicity factors is complex and involves transcriptional and post-transcriptional regulation processes (García-Del Portillo & Pucciarelli, 2017; Smith *et al.*, 2016; Shen e Fang, 2012). One example of a complex regulation process is the operon *flj*BA, responsible for encoding flagellar proteins, that is mediated by a post-transcriptional mechanism. This operon contains two flagellin genes *fliC* and *fliB* that can be alternatively expressed in some serovars such as *S.* Typhimurium. The gene *fljB* is cotranscribed with the gene *fljA* which inhibits *fliC* at transcriptional and translational level stopping it to produce the FliC protein. The flagellar phase variation depends on the orientation of the DNA fragment *hin.* This fragment contains *fljB* and *fljA* promoters besides a gene that encodes a recombinase Hin. This recombinase will catalyze the inversion of this fragment, resulting in two different possible orientations to the *hin* sequence and consequently the expression of two possible flagellins. The *flj*BA^{ON} orientation will result in the expression of FliB protein and the *flj*BA^{OFF} will result in the expression of FliC

(Bonifield & Hughes, 2003). The flagellar phase variation has shown to be an important characteristic of *S.* Typhimurium pathogenicity, with mutants expressing only *fljB* showing attenuation in mice model (Ikeda *et al*., 2001).

Nonetheless, the isolation of *S. enterica* monophasic variant (I 4,[5],12:i:-), a Typhimurium variant that only expresses one type of flagellin, has been described in all continents, and has been associated with antimicrobial resistance and severe infections in humans and other animals worldwide (Switt *et al.*, 2009). This serotype lacks the flagellar phase variation ability, and phenotypical and molecular analysis indicated it was originated from *S. enterica* Typhimurium (Echeita *et al.*, 2001; de la Torre *et al.*, 2003; Kurosawa *et al.*, 2012). Different deletion patterns of the *fljBA* operon, more likely to have occurred by independent events, are associated with the monophasic variant origin with three different clones being described, the Spanish (S), the European (E), and the United States (U.S.) (Garaizar *et al.*, 2002, Soyer *et al.*, 2009, Bugarel *et al.*, 2012, Barco *et al.*, 2014, Boland *et al.*, 2015). This serotype has been among the most isolated ones in Spain (Guerra *et al.*, 2000) and in the United States (CDC, 2011). In Brazil, the monophasic variant has been isolated since 1990 and it has been associated with human-infections causing gastrointestinal problems as well as systemic infections (Taunay e*t al*., 1996; Tavechio *et al.*, 2004).

3.3. Epidemiology

S. enterica causative agent of Salmonellosis is one of the most prevalent pathogens associated with foodborne infections worldwide. Contaminated products derived from poultry, livestock, and swine are among the most common source of this foodborne disease (Crump et al., 2015). The invasive S. enterica serotypes cause in mammals infections with different degrees of severity since gastroenteritis in the intestinal mucosa to severe systemic infections.

3.3.1. Enteric Fever

Enteric fever or typhoid fever, a systemic infection that affects humans, is caused by typhoidal *Salmonella,* which includes the serotypes Typhi and Paratyphi. Although Salmonellosis occurs worldwide, typhoid fever presents higher morbidity and mortality in developing countries with mortalities rates achieving 7% despite the availability of antimicrobials (Eng et al., 2015). In 2000 the World Health Organization (WHO) estimated 16 to 33 million typhoid fever cases worldwide with 500 to 600 thousand fatalities, while paratyphoid fever cases affected 5.4 million people (Woc-Colburn e Bobak, 2009). More

recent data released by the International Vaccine Institute in 2010 estimates 11.9 million people being affected by typhoid fever with 129,000 fatal cases just in low- and middle-income countries (Crump et al., 2015).

The African and Asian continents are the most affected by typhoid and paratyphoid fever. While in the USA and some European countries the typhoid fever incidence is around 10 cases per 100,000 individuals annually, some Asian and African counties surpass 100 cases per 100,000 habitants (Eng et al., 2015). Enteric fever does not affect all African and Asian countries in the same way. A study conducted in Africa compared 13 different regions where enteric fever cases have been reported, and the typhoid fever rates per 100,000 habitants reached 383 in some locations with individuals younger than 15 years old being the most affected (Marks et al., 2017). Among Asian countries, Pakistan and India present the highest incidence of enteric fever with more than 200 cases per 100,000 population, but when considering only children below five years old the enteric fever rates reached 450 per 100,000 every year.

In addition to the typhoid fever cases, Asian countries also present high prevalence of paratyphoid fever. For instance, in Israel the serovar Paratyphi is the causative agent of 57.4% cases of enteric fever, and in Asian countries in general paratyphoid fever represents 50% of enteric fever cases (Eng et al., 2015). However, the cases are still underreported and the differences in data availability and incidence studies jeopardize the epidemiological studies.

In Latin America data gaps about enteric fever remains a problem to be overcome. A global systematic review conducted in 2010 estimated 120 cases per 100,000 population in Latin America, but there was no data about paratyphoid fever nor data about the incidence of enteric fever in infants, which presents a challenge to epidemiologists trying to understand the incidence and severity of this disease (Buckle et al., 2012).

3.3.2. Non-typhoidal *Salmonella enterica*

The virulence of non-typhoidal *Salmonella* is associated with the serotype and with the host being affected (Crump *et al.*, 2015). While serovars Typhi and Paratyphi are the major causative agents of systemic infections in humans, the mainly etiological agents responsible for septicemia in swine and bovine are *S. enterica* Choleraesuis and *S. enterica* Dublin, respectively. Although these serotypes affect these animals, they can also cause acute infections in humans (Andrews-Polymenis *et al.*, 2010; Mastroeni & Maskell, 2006).

The most prevalent serovars among NTS infections in humans are *S. enterica* Typhimurium and Enteritidis, and they are responsible for causing gastrointestinal and extraintestinal infections worldwide (Hendriksen *et al.*, 2011). For instance, in Latin America *S.* Enteritidis accounts for 37% of NTS human-infections, while in Europe almost 87% of cases are related to this serotype (Eng et al., 2015). These serovars are also associated with non-human infections causing in mice a similar systemic infection to typhoid fever in humans. Therefore, some mice lineages are used as the murine model to study systemic infections caused by *S. enterica* (Verma & Srikanth, 2015; Tsolis *et al.*, 2011; Watson e Holden, 2010). Among the *S. enterica* serovars, some present host's specificity causing infection only to that host, while others are generalists and can infect mammals, birds, reptiles, amphibians, and plants (Wiedemann *et al.*, 2015; Hernández-Reyes e Schikora, 2013).

Furthermore, Salmonellosis caused by non-typhoidal serovars (NTS) is frequent globally (Ao *et al.*, 2015; Crump *et al.*, 2015; Andrews-Polymenis *et al.*, 2010). In a meta-analysis study, Reddy *et al.* (2010) observed that one of the main causative agents of septicemia in Africa is *S. enterica* particularly non-typhoidal serotypes denominated *invasive Non-typhoidal Salmonella* (iNTS). Recently, review articles about iNTS have been published showing the global occurrence of them with 3.4 million annual cases and 680 thousand fatalities (Crump *et al.*, 2015; MacFadden *et al.*, 2016).

In Brazil, from 1999 to 2008 approximately 6602 outbreaks caused by enteric pathogens have occurred, and 43% of these cases were correlated to *S. enterica* (Medeiros *et al.*, 2011). The Brazilian government took sanitary measures in 2014, before the world cup, evaluating the most prevalent foodborne pathogens between 2000 and 2013, and then ellaborating strategies to mitigate foodborne infections. According to those reports around 9000 foodborne diseases outbreaks have happened during the evaluated period, and 39% of them were caused by *S. enterica* (Ritter e Tondo, 2014). Additionally, other research studies have reported the high prevalence of *Salmonella enterica* as a foodborne pathogen, for instance in a study performed in the state of São Paulo, 3554 samples of *Salmonella enterica* were isolated from human infections during the period of 1996 until 2003, and the isolates showed a great diversity with approximately 68 different serotypes being detected (Fernandes *et al.*, 2006).

Salmonellosis still a great cause of concern globally not only because of its high incidence and severity but also for the increasing number of multiresistant strains that make treatment harder (MacFadden *et al.*, 2016; Crump *et al.*, 2015). For instance, the acquisition

of antimicrobial resistance genes (AMR) by *S. enterica* Typhimurium has been shown to play an important role in its evolution because AMR genes can be located in Genomic Islands (GIs) that also carry fitness-associated genes to the bacteria presenting an adaptive advantage (Paul *et al.*, 2016).

3.3.3. Non-*enterica* subspecies of *Salmonella enterica*

Even though human Salmonellosis is mostly caused by *S. enterica* subspecies *enterica,* non-*enterica* subspecies have been isolated from clinical samples (Lamas et al., 2018). *Salmonella* spp. is known for infecting a wide range of hosts, while *S. enterica* is more often linked to infections in warm-blooded animals, the non-*enterica* subspecies are more common in cold-blooded animals and the environment. Because of the clinical relevance of subspecies *enterica* pathogenicity, the epidemiological data of Salmonellosis are focused on *enterica* subspecies while there are not as much data about non-*enterica* infections. Most of the non-*enterica* cases reported in humans are linked to immunocompromised individuals or infants, but asymptomatic cases in healthy humans have also been described (Lamas et al., 2018; Hervas et al., 2012).

Reptiles and amphibians are known as a source of *Salmonella* spp. infections in humans, with cases being linked to *enterica* and non-*enterica* subspecies. A case-control study published in 2004 estimated that 74,000 cases of human infections are caused by a reptile or amphibian exposure (Mermin et al., 2004). The first report of human Salmonellosis linked to contact with contaminated reptiles was in the United States in 1963 (Ward, 2000). *Salmonella* spp. is part of the reptiles and amphibians microbiota; thereby they do not present infection symptoms, but they can transmit the pathogen through contact or by eating their meat without proper cooking (Lamas et al., 2018). Although *S. enterica* can be part of those animals microbiota, studies have shown the high prevalence of subspecies *diarizonae, arizonae, salamae* and *houtenae* among wild, zoo and pet reptiles (Lamas et al., 2018; Schröter et al., 2004; Nowakiewicz et al., 2012; Geue & Loschner, 2001).

Exotic pets, such as reptiles and amphibians, are becoming more popular since the late 1990s in Europe and the United States. The increasing number of exotic pets is being linked with the increasing numbers of Salmonellosis in exotic pet owners or people that had contact with those animals. Children are the most affected ones because while healthy humans can be asymptomatic carriers, young children can develop meningitis, gastroenteritis, or sepsis (Ward, 2000). For instance, there were reports of *S. diarizonae* isolated from gastroenteritis infections and cerebrospinal fluid in neonates from different continents (Hervas et al., 2012;

Giner-Lamia et al., 2019). A study in French Guiana correlated strains of *S. houtenae* isolated from reptiles with human infections caused by the same strains (Gay et al., 2014).

Moreover, a surveillance study conducted in Europe in 2008 reported cases of human Salmonellosis (caused by non-*enterica* subspecies) associated with reptiles in the Netherlands, Ireland, Germany, Belgium, and Finland indicating non-*enterica* subspecies can infect humans (Bertrand et al., 2008). Recent cases have been published with fatal outcomes in Bolivia and Germany, both cases linked to *S. diarizonae* belonging to different serotypes. In Bolivia, the bacterial infection isolated from cerebrospinal fluid affected a newborn, while in Germany it caused diarrhea and sepsis in an adult. Even though neither cases were reported as linked to reptile or amphibians contact, it shows evidence other *Salmonella* subspecies can be invasive to humans (Giner-Lamia et al., 2019; Gerlach et al., 2017). Therefore, more studies about the pathogenicity and incidence of these subspecies need to be conducted.

3.4. Antimicrobial Resistance in *Salmonella* spp.

3.4.1. Typhoidal *Salmonella*

The treatment against *S. enterica* infection is becoming more challenging due to the increasing number of bacteria carrying antimicrobial resistance genes correlated with clinically important drugs (Crump *et al.*, 2015). In the case of typhoidal-Salmonellosis treatment, chloramphenicol used to be the drug of choice in 1970 (Crump *et al.*, 2015). However, in the following years there were outbreaks of typhoid fever caused by chloramphenicol-resistant strains in different countries (Rowe *et al.*, 1997). In most cases correlated to this outbreak the resistance present among the strains was caused by a mobile element, a plasmid from the incompatibility group IncHI that also carried other resistance genes (Rowe *et al.*, 1997; Anderson, 1975).

The most prevalent resistance mechanism among resistant-strains is the production of acetyl-transferases (CATs). Nevertheless, there are other non-enzymatic resistance mechanisms associated with chloramphenicol resistance in *Salmonella* spp, such as the expression of *cmlA* e *floR* genes (Crump *et al.*, 2015). The gene *floR* is correlated with florfenicol-chloramphenicol resistance, while gene *cmlA* only results in chloramphenicol resistance. Both genes encode efflux pumps proteins that export the antimicrobials out of the cell (Braibant et al., 2005; Mendonca, 2016).

The rise of chloramphenicol-resistant bacteria resulted in increased use of other antimicrobials such as ampicillin and trimethoprim/ sulfamethoxazole (Crump *et al.*, 2015).

Nonetheless, at the end of 1980s, multi-drug resistance strains (MDR) started to be isolated showing resistance against ampicillin, chloramphenicol, and trimethoprim/ sulfamethoxazole, and this MDR pattern is still commonly isolated (Feasey *et al*., 2015; Tatavarthy *et al.*, 2014; Wain *et al.*, 1999). The resistance against ampicillin is usually mediated by β-lactamases production (bla_{PSE} and bla_{TEM}), while trimethoprim/ sulfamethoxazole-resistance is caused by the expression of genes (*dfr*, *sul1* e *sul2*) controlling folate metabolism that is not inhibited by this drug (Crump *et al.*, 2015).

The use of fluoroquinolones, such as ciprofloxacin, became the alternative to treat typhoid fever caused by MDR strains. However, there are reports of *S.* Typhi and Paratyphi presenting resistance against this antimicrobial class (Crump *et al.*, 2015), and the number of isolates with a decreased susceptibility to other fluoroquinolones and quinolones have been rising due to strains presenting mutations in DNA gyrase (fluoroquinolones/quinolones target), or carrying Plasmid-Mediated Quinolone Resistance (PMQR) genes (Sjölund-Karlsson *et al.*, 2014; Demczuk *et al.*, 2010; Threlfall & Ward, 2001).

More recently, azithromycin, azalides, and broad-spectrum cephalosporins were incorporated as a treatment against MDR typhoidal-*Salmonella*. Nevertheless, resistant strains carrying β-lactamases genes encoding proteins such as SHV-12, CTX-M, and AmpC have been isolated (Ahmed *et al.*, 2012; Gokul *et al.*, 2010), as well as strains resistant to azithromycin (Jain e Chugh, 2013).

3.4.2. Non-typhoidal *Salmonella* spp.

Likewise typhoidal-*Salmonella* strains, the evolution of MDR isolates have also been described for non-typhoidal *Salmonella* (NTS) strains. MDR Typhimurium samples were isolated during the 1980s in the United Kingdom, and they were associated with a specific phage-type denominated DT104 (Threlfall, 2002). Subsequently, MDR NTS samples were isolated in other countries around the globe (Reis *et al.*, 2011; Medalla *et al.*, 2017; MacFadden *et al.*, 2016; Parry, 2003; Threlfall, 2000).

One MDR pattern commonly distributed among NTS samples presents resistance against ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (R-type ACSSuT). This resistance pattern is mediated by a chromosomal element denominated *Salmonella* Genomic Island 1 (SGI-1) (Kiss *et al*., 2012; Mulvey *et al.*, 2006). The original structure of SGI-1 is composed by a 14kb element divided into two integrons containing the resistance genes bla_{PSE-1} (ampicillin), *floR* (chloramphenicol and florfenicol), *aadA2* (streptomycin), *sul1* (sulfonamide) e *tetG* (tetracycline) (Kiss *et al*., 2012).

Another problem that aggravates the treatment against *Salmonella enterica* is the presence of quinolones and fluoroquinolones resistance strains worldwide (Crump *et al.*, 2015). The resistant phenotype found among NTS is correlated with mutations within genes encoding the target enzyme, such as mutations at the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), called QRDR regions (quinolone resistance-determining regions), or through the plasmidial genes *qnrb-2* and *qnrS-1* (Crump *et al.*, 2015).

The β-lactams antimicrobial class is the treatment choice against quinolone/fluoroquinolone-resistant strains. However, the resistance against broad-spectrum β-lactams has been reported since 1980 in different countries. This resistance is associated with the production of extended-spectrum β-lactamases, and the most common genes correlated with this phenotype in NTS are bla_{CMY} and bla_{CTX}. This scenario is aggravated by the increasing number of MDR NTS, presenting resistance against three to seven different antimicrobial classes (Crump *et al.*, 2015).

Another great cause of concern is the emergence of colistin-resistant NTS. Even though colistin is the last resource drug to treat bacterial infections, it is still used in veterinary medicine in many countries, which could be correlated with the emergence of resistant strains in human infections. For instance, Litrup et al. (2017) have reported ten *Salmonella enterica* isolates harboring the *mcr-3* gene, responsible for the colistin-resistance phenotype, in human infections over the period of 2009 to 2017. Moreover, one of the samples co-harbored *mcr-1* and *mcr-3* in the same plasmid. The *mcr* genes are located in mobile elements facilitating the dissemination of them among bacterial cells, thereby the emergence of colistin-resistant NTS harboring plasmidial genes represents a threat to public health and measures need to be taken to mitigate the dissemination of such genes (Apostolakos *et al.,* 2018).

In contrast, there are no reports of non-*enterica Salmonella* spp. isolated from humans with a resistant phenotype (Gerlach et al., 2017; Gay et al., 2014; Hervas et al., 2012; Bertrand et al., 2008). However, there are still limited studies and reports on these subspecies limiting the information about their ability to acquire resistance genes. Among non-human isolates, there is also a low prevalence of resistant strain. However, a study conducted with Russian tortoises in 2012 isolated *S. salamae* presenting resistance against amoxicillin and intermediate sensibility to colistin, and in this same group of animals that were *enterica* subspecies with the same susceptibility profile showing the spread of resistance genes are possible among different subspecies (Nowakiewicz et al., 2012). Moreover, *Salmonella arizonae* isolated from pork meat presented resistance against sulfamethoxazole-

trimethoprim, tetracycline, ampicillin, and amoxicillin. These antimicrobials are commonly used in food production in certain countries which could have driven the selection of resistant strains (Evangelopoulou et al., 2014). While in Australia 80.4% of *Salmonella salamae* serovar Sofia isolated from food-producing animals presented resistance only against streptomycin. Generally, most studies conducted with non-*enterica* species isolated from animals present a high sensitivity to antimicrobials (Lamas et al., 2018).

4. Materials and Methods

4.1.Bacterial Isolates

All bacterial isolates were provided by Instituto Adolfo Lutz (IAL) - Centro de Laboratório Regional de Campinas III and by Hospital Universitário da USP- São Paulo. The samples provided by IAL-Campinas were isolated and identified by them and sent afterward to IAL-São Paulo for serotyping. In the same manner, samples from HU-USP were isolated and identified by the microbiology laboratory located within the Hospital, then sent to IAL-SP for serotyping. After isolation, identification, and serotyping all samples were stored in IAL-Campinas and HU-USP sample collection from 2000 to 2019.

The isolates from the sample collection of both places were re-isolated and their genus *Salmonella* spp. was confirmed again through biochemical tests to ensure all isolates were still pure. In total 810 samples were stored at -80ºC and selected for this study. Appendix 01 shows the serovars' diversity and prevalence among the 810 isolates and their respective antigenic formulae. Appendix 02 shows the number of isolates corresponding to each source of isolation and Appendix 03 shows the total number of isolates per year. Table 04 presents the five most prevalent serovars among our samples and the number of isolates from epidemiologically important serovars herein studied.

Table 04. *Salmonella* spp. serovar's diversity of samples collection from IAL-Campinas and HU-USP-São Paulo used in this study.

Serovar	**Nº of Isolates**	
	N	**%**
Enteritidis	360	44.44
Typhimurium	79	9.75
Monophasic	66	8.15
Dublin	30	3.70

Saint Paul	29	3.58
Typhi	15	1.85
Paratyphi	3	0.37
***subspecies diarizonae* ser. 61**	2	0.25
***subspecies salamae* ser. 42**	1	0.12
not-determined	70	8.64
Others	156	21.85
Total	810	100

This project was approved by the ethics committee from the host institution UNICAMP under the CAAE number 91276318.2.0000.5404, and from the co-participants institutions IAL under the number 91276318.2.3001.0059 and USP under the number 91276318.2.3002.0076. The ethics committee did not require the informed consent from patients where the bacterial samples were isolated. Additionally, following the ordinance SECEX/CGEN nº 1 from October 3rd, 2017, samples herein studied were also registered in SisGen ("Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado").

4.2. Antimicrobial Resistance Testing

4.2.1. Disk Diffusion Assay

The antimicrobial resistance profile of the 810 samples was evaluated using the Kirby-Bauer disk diffusion susceptibility test following the CLSI M02-A12 (CLSI, 2015) instructions. This assay was performed using the Mueller-Hinton agar (Oxoid or Difco), and the choice of antimicrobials, as well as the interpretation of the inhibition halos, were made based on the CLSI M100 document (CLSI M100, 2020). The following antimicrobials discs were chosen according to CLSI M02-A12 recommendations: amoxicillin (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), meropenem (10 µg), ertapenem (10 µg), imipenem (10 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), tetracycline (30 µg), streptomycin (10 µg), gentamicin (10 µg), trimethoprim/ sulfamethoxazole (25 µg) and colistin (10 µg). The inhibition zones were measured after 18h incubation at 37ºC. The quality control of the test was performed using the reference strain *Escherichia coli* ATCC 25922, sensitive to all antimicrobials herein tested.

4.2.2. ESBLs and AmpC Phenotypic Detection

The modified double-disk synergy test was done to detect the production of type AmpC beta-lactamases (Ruppé et al., 2006), where cefoxitin/cloxacillin (30 µg / 200 µg) disks were used as previously described (Thean et al., 2009). Samples resistant to cefoxitin in the Kirby-Bauer test were submitted to this analysis.

Samples resistant to third-generation cephalosporins in the Kirby-Bauer test were submitted to the ESBL-producer confirmatory test described in CLSI M100 (CLSI M100, 2020). In summary, cefotaxime (30 µg) and ceftazidime (30 µg) (Oxoid) without inhibitors, as well as combined with clavulanic acid (10 µg), were used for the detection. Samples were considered positive when the inhibition zones of the cephalosporin disks with clavulanic acid were ≥ 5 mm compared to the zones formed by disks without inhibitors.

Samples resistant to imipenem (≤ 22 mm) and/or meropenem (≤ 24 mm) were submitted to additional tests in order to detect carbapenemase production. Inhibition zones of meropenem and imipenem with and without the addition of EDTA (0.1 M), cloxacillin (75 mg/ml), and phenylboronic acid (40 mg/ml) were compared. The results were interpreted following the ANVISA (2013) document, where isolates with zones of inhibition ≥ 5 mm for the disks with EDTA were considered metalo-beta-lactamase producers. The isolates that presented zones of inhibition ≥ 5 mm for the disks with phenylboronic acid were considered carbapenemase-producers (KPC), and those with zones of inhibition ≥ 5 mm for cloxacillin and phenylboronic acid were considered AmpC-producers.

During all the ESBLs' detection tests samples were prepared following the same instructions of the Kirby Bauer test. 10 µl of the inhibitors were added to the respective disks and after 20 minutes they were added to the plate containing the sample in Mueller Hinton agar, afterward, plates were incubated at 37^{o} C for 18 hours. The reference strains *K. pneumoniae* ESBL ATCC 700603 and *Escherichia coli* 25922 were used as quality controls.

4.2.3. Minimal Inhibitory Concentration (MIC)

In order to detect samples that were colistin-resistant we first performed the Kirby-Bauer test using colistin disks as a first screening of the 810 samples. Isolates that presented a zone of inhibition ≤ 10 mm, which is considered non-susceptible for *Enterobacteriaceae* bacteria (CLSI, 2020), were submitted to the Minimal Inhibitory Concentration (MIC) test. As established by the Clinical Laboratory Standards Institute the MIC test is the

recommended one to detect colistin resistance due to the low diffusion of colistin in Mueller Hinton (MH) agar.

As described in the CLSI M07-A10 document (CLSI M07-A10, 2015) the bacterial cultures, cultivated in MH agar one day before the test, were suspended in MH broth to achieve the concentration of 10^5 CFU/ml. The bacterial suspensions (100 µl) were distributed in 96 wells plates containing 100 µl of cation supplemented MH broth with colistin serial dilutions (0.25 µg/ml – 32 µg/ml). The microdilution plates were incubated at 37ºC for 18 hours and the MIC results interpreted following the CLSI M100 document (CLSI M100, 2020), where samples were considered resistant when MIC >2 µg/ml. This assay was repeated three independent times and the reference strain *E. coli* ATCC 25922 was used as quality control.

Furthermore, samples considered resistant in the MIC had their Minimum Bactericidal Concentration (MBC) tested by subculturing samples that did not present a visible growth in the microdilution plates (colistin concentrations >2 µg/ml) onto agar plates without any antimicrobial agent. The MBC value was considered as the colistin concentration that did not result into bacterial growth in the agar plate after 18 hours of incubation at 37ºC (CLSI M26-A, 1999).

4.3.Whole Genome Sequencing and Bioinformatics Analysis

In order to understand better the antimicrobial resistance genotype and virulence factors of our samples we selected for whole-genome sequencing the three subspecies non-*enterica* isolates, which are not commonly reported in human-infections (*S. salamae* 644/10, *S. diarizonae* 08/16 and 30/10), and we also selected three isolates of *S.* Typhi from an outbreak in 2006 in order to better characterize the isolates involved in it (Typhi 303/06, 328/06, and 385/06). Moreover, among the 71 samples that presented MDR resistance phenotype we selected the isolate Enteritidis 520/08 which was the only one to present carbapenem resistance, the monophasic variant 725/16 which was the only monophasic to present fluoroquinolone resistance, the Typhi 13/11 for being an MDR typhoid isolate presenting beta-lactam resistance, and the Infantis 14/05 the only MDR sample from this serovar which is a serovar that has been poorly characterized in Brazil, but it has been recently reported in several cases of human-infections and food-production animals in Europe. The bacterial isolates' DNA was extracted using a commercial kit following the manufacturer's guidelines (Wizard® Genomic DNA Purification/ Promega/ United States).

The Nextera™ XT DNA kit (Illumina Inc., Hayward, CA) was used to prepare the DNA library. The genomic DNA of our samples were sequenced using a 250-bp paired-end-read strategy in the Illumina HiSeq2500 (Illumina Inc., San Diego, CA) platform at the Central Laboratory of High Perfomance Technologies (LaCTAD).

The resulting reads were assembled using SPAdes v3.11.1 (Bankevich et al., 2012) with the collaboration of bioinformaticians at LaCTAD and the National Laboratory of Scientific Computation (LNCC). The resulting contigs were annotated using the NCBI prokaryotic genome automatic annotation pipeline (PGAAP). In order to confirm the serovars of our samples SeqSero 1.0 (Zhang et al., 2015) was used, and to determine the sequence type of each isolate the MLST (Multi-Locus Sequence Typing) 2.0 software (Larsen et al., 2012) was chosen (available at http://cge.cbs.dtu.dk/services/MLST/). The Typhimurium monophasic variant was confirmed aligning the operon sequence from the reference strain *S.* Typhimurium LT2 with our isolate in order to detect deletions in the operon *fljBA* using BLASTn and MAUVE 2.1 (Darling et al., 2004).

The antimicrobial resistance-associated genes were investigated using CARD V3.0.8 (Alcock et al., 2020) and ResFinder 3.1 (Zankari et al., 2012) using default settings (≥95% identity) and confirmed afterward using BLASTn. The contigs were submitted to PlasmidFinder 2.1 (Carattoli et al., 2014) to detect the presence of plasmids using a threshold of 95% of identity and 60% of minimum coverage, the replicon types found had their plasmid MLST determined by pMLST 2.0 (Carattoli et al., 2014). PHASTER (Arndt et al., 2016) was used to detect the presence of intact phage sequences using default parameters. Virulence factor sequences were determined by the Virulence Factor Database (VFDB 2019) (Liu et al., 2018) and compared to our samples and reference strains available at the software.

The presence of intact *Salmonella* Pathogenicity Islands (SPI) was determined by SPI-Finder 1.0 online tool (available at https://cge.cbs.dtu.dk/services/SPIFinder) using a ≥ 95% identity and ≥ 60% minimum length. In order to confirm the presence of the most important SPIs (SPI-1 through SPI-6) their sequences were obtained from *S.* Typhimurium LT2 and aligned with our isolates using BLASTn and MAUVE 2.1 (Darling et al., 2004).

Plasmid sequences were obtained from NCBI, and BLASTn was used to compare reference plasmids found with the same mobile elements and resistance genes of sample 725/16. Selected plasmid references were aligned with our sample using default parameters in MAUVE 2.1 (Darling et al., 2004).

4.4.*Galleria mellonella* Killing Assay

Laboratories of the University of São Paulo (USP) – Ribeirão Preto, and USP- São Paulo donated *Galleria mellonella* eggs and the larvae were bred in our laboratory. Larvae presenting no signs of illnesses, no melanization, and weighing approximately 250 mg were selected for the experiment.

The subspecies non-*enterica* isolates 08/16 (*S. diarizonae*), 30/10 (*S. diarizonae*), and 644/10 (*S. salamae*) were selected to *in-vivo* tests using *Galleria mellonella* to compare their virulence. The reference strain *Salmonella* Typhimurium UK-1 (provided by Professor Roy Curtis III, College of Veterinary Medicine, University of Florida, USA) was used as the virulent control, the *Escherichia coli* OP50-1, a strain used to *Caenorhabditis elegans* growth (provided by Professor Marcelo Alves da Silva Mori, Institute of Biology, University of Campinas, Brazil), was used as the non-virulent control, and phosphate-buffered saline (PBS-10 mM) as the negative control.

All bacteria strains were grown overnight in Luria-Bertani (LB) broth at 37ºC. On the next day, all isolates were transferred to a new media (LB) and grown at 37ºC and 220 rpm until they have reached the proximal concentration of 10^8 CFU/ml (optical density of ~0.9 at 600 nm). Afterward the isolates were centrifuged and washed with PBS, and then serial dilutions were made to obtain the desired concentrations for the inoculum. The dilutions were plated in LB agar to determine the CFU of samples; plates were then incubated at 37ºC for 18h.

The non-virulent control *E. coli* OP50-1 was injected using the concentrations of 10^6 CFU/µl and 10^5 CFU/µl, while all the other bacterial strains dosages were 10^4 CFU/ µl, 10^3 CFU/µl, and 10^2 CFU/µl. The volume of 10 µl of each bacteria was used for the injection into the larvae last left proleg, the same volume was used for the control larvae (PBS) to make sure there were no problems with the injection process. Groups of 10 larvae were injected for each strain tested and 5 larvae for the PBS control-group, and afterward they were placed in Petri dishes at 37ºC. Their survival was followed, according to their appearance and response to stimuli, during 96h. Larvae that did not respond to touch were considered dead. The experiment was repeated three independent times, and the representative results were displayed as percent survival in a Kaplan-Meier curve.

4.5.Macrophage Invasion and Survival

J774 macrophages cells were cultured overnight in 96 wells plates at 37ºC and 5% CO_2 in order to achieve 95-100% confluence in RPMI medium. The subspecies non-*enterica* samples were used for this assay and were compared to the reference strain *Salmonella* Typhimurium ATCC 14028 which is known for being capable of invade and survive within J774 macrophages. The bacterial isolates were grown overnight in LB broth at 37 ºC and 220 rpm for 18h. The overnight bacterial cultures were then inoculated into a new LB broth and incubated at 37ºC and 220 rpm until they reached the concentration of 10^8 CFU/ml (optical density of ~0.9 at 600 nm). Afterward, the bacterial cultures were inoculated into the overnight J774 culture plates with an MOI (multiplicity of infection) of approximately 20 bacteria per macrophage. Each strain was inoculated in three different wells, and two plates were used during the assay; one to the invasion assay and another one to survival. After inoculation, the plates were briefly centrifuged to facilitate the bacteria contact with the macrophages following the incubation at 37ºC with 5% CO_2 for one hour. Non-phagocytosed bacteria were then removed by washing the plates with RPMI containing gentamicin (100 µg/ml). One plate was incubated for another hour to follow cell invasion while a second plate was incubated for 5 hours to check intracellular survival. Both plates were incubated in RPMI with gentamicin (20 µg/ml) at 37ºC and 5% CO_2. After the respective incubation period time, the plates were washed with PBS, following the cell lysis using 0.1% Triton X-100 in PBS. The number of intracellular bacteria was counted by plating aliquots of the lysed cells, after serial dilutions, onto LB agar plates.

5. Results

5.1. Antimicrobial Resistance Profile

5.1.1. Disk Diffusion Assay

Table 05 shows the percentage and number of resistant isolates for each drug tested. The first four columns present the resistance of the most prevalent serovars. Moreover, the resistance profile of typhoid (Typhi and Paratyphi) and non-*enterica* (*S.* subsp. *diarizonae* and *salamae*) isolates are also shown. The percentage of those columns was calculated considering as 100% the total number of isolates for each serovar of the correspondent column. The last column shows the percentage and number of resistant isolates in total, considering as 100% the 810 isolates used in this study.

Among the 810 samples tested, 355 were resistant to at least one antibiotic. The isolates herein studied presented a higher percentage of resistance against streptomycin

(29.28%), tetracycline (13.44%) and amoxicillin (12.36%). The serovars that presented the highest number of resistant samples were the three most prevalent serovars among the total samples, with 24.79% of resistant samples corresponding to the serovar Enteritidis, followed by 16.62% of serovar Typhimurium and 11.55% of the monophasic variant.

Regarding the Enteritidis isolates 11.42% were resistant to amoxicillin, 9.47% to streptomycin and 7.24% to tetracycline. One of the isolates was resistant to third-generation cephalosporins herein tested and to a carbapenem (meropenem). This Enteritidis isolate was the only among the 810 to present resistance against carbapenems. Moreover, six isolates from this serovar were considered as possibly resistant to colistin (with an inhibition halo ≤10 mm).

Samples from the Typhimurium and monophasic variant serovars presented a similar antimicrobial resistance profile, only diverging in beta-lactam resistance profile. Likewise Enteritidis, both serovars presented a higher percentage of resistance against streptomycin, amoxicillin, and tetracycline. Moreover, both serovars also presented possible resistance to colistin in the Kirby-Bauer test. Nevertheless, regarding resistance to cephalosporins, there were differences. Three Typhimurium isolates were resistant to third-generation cephalosporins, while two monophasic variant isolates were resistant only to a second-generation cephalosporin.

Among the typhoid isolates (15 Typhi and 3 Paratyphi), 55.56% were resistant to streptomycin, 22.22% to sulfamethoxazole/trimethoprim, and only 16.67% to amoxicillin, while only one Typhi isolate was resistant to tetracycline, which shows a slight different antimicrobial resistance profile when compared to the non-typhoidal serovars. All typhoid isolates were sensitive to cephalosporins, carbapenems, fluoroquinolones, polymyxin B, and chloramphenicol showing a susceptible resistance profile.

The two isolates of *S. diarizonae* and the only *S. salamae* isolate, classified as non-*enterica* isolates in Table 05, were resistant to streptomycin and sensitive to all the other drugs tested.

Table 05. Percentage and absolute number (N) of antimicrobial resistance among the isolates herein studied.

	Enteritidis	Typhimurium	Monophasic	Dublin	Typhoid	non-*enterica*	**Total S**
	% (N)	% (N)	% (N)	% (N)	% (N)	% (N)	% (N)
Amoxicillin	11.42% (41)	21.52% (17)	21.21% (14)	20% (6)	16.67% (3)	0% (0)	12.72% (103)
Cefotaxime	0.28% (1)	2.53% (2)	0% (0)	0% (0)	0% (0)	0% (0)	0.62% (5)
Cefoxitin	0% (0)	0% (0)	3.03% (2)	3.33% (1)	0% (0)	0% (0)	0.37% (3)
Colistin	1.67% (6)	2.53% (2)	4.55% (3)	6.67% (2)	0% (0)	0% (0)	2.1% (17)
Ciprofloxacin	0.28% (1)	1.27% (1)	1.52% (1)	0% (0)	0% (0)	0% (0)	0.37% (3)
Chloramphenicol	2.23% (8)	16.46% (13)	15.15% (10)	3.33% (1)	0% (0)	0% (0)	5.06% (41)
Ceftazidime	0.28% (1)	1.27% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0.49% (4)
Enrofloxacin	2.79% (10)	5.06% (4)	3.03% (2)	6.67% (2)	0% (0)	0% (0)	3.95% (32)
Ertapenem	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Iminipenem	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Gentamycin	4.18% (15)	11.39% (9)	16.67% (11)	6.67% (2)	5.56% (1)	0% (0)	6.17% (50)
Streptomycin	9.47% (34)	56.96% (45)	46.97% (31)	43.33% (13)	55.56% (10)	100% (3)	30.12% (244)
Meropenem	0.28% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0.12% (1)
SXT[1]	2.51% (9)	17.72% (14)	12.12% (8)	10% (3)	22.22% (4)	0% (0)	6.42% (52)
Tetracycline	7.24%(26)	26.58% (21)	28.79% (19)	40% (12)	5.56% (1)	0% (0)	13.83% (112)
Total T	100% (360)	100% (79)	100% (66)	100% (30)	100% (18)	100% (3)	100% (810)

[1]SXT: sulfamethoxazole/trimethoprim

Table 06 shows the percentage of resistant samples according to each antimicrobial class herein tested. The majority of samples were resistant to aminoglycosides (32.10%), followed by tetracyclines (13.83%) and beta-lactams (13.21%). Among the isolates resistant to fluoroquinolones and/or beta-lactams the serovars Typhimurium and Enteritidis were the most prevalent.

Table 06. Percentage and absolute number (N) of samples classified as non-susceptible (NS) and susceptible (S) to each antimicrobial class herein evaluated.

	NS		S	
	%	N	%	N
Beta-Lactams	13.21%	107	86.79%	703
Colistin	2.10%	17	97.90%	793
Chloramphenicol	5.06%	41	94.94%	41
Fluoroquinolones	4.07%	33	95.93%	777
Aminoglycosides	32.10%	260	67.90%	550
SXT[1]	6.42%	52	93.58%	758
TE[2]	13.83%	112	86.17%	698

[1]SXT: Sulfamethoxazole/trimethoprim [2]TE: Tetracycline

Within the 355 samples that presented resistance to at least one antibiotic, 71 were resistant against three or more antimicrobial classes simultaneously. Those samples are classified as multidrug-resistant (MDR). Figure 01 shows the MDR patterns found in our isolates, besides their serovars and source of isolation.

The most common MDR pattern found was beta-lactams, aminoglycosides, tetracycline (B+A+T) with 23 isolates presenting this pattern. Patterns commonly described in *Salmonella* spp. such as ACSSuT (ampicillin-amoxicillin, chloramphenicol-florfenicol, streptomycin-spectinomycin, sulfonamides, and tetracycline) were found only in six isolates, represented by B+C+A+S+T (beta-lactams, chloramphenicol, aminoglycosides, sulfamethoxazole/trimethoprim, tetracycline) in Figure 01. The serovars with the highest number of MDR samples were Typhimurium followed by monophasic variant and Enteritidis (Figure 01-C). The most common sources of MDR isolates were feces, blood, and urine (Figure 01-B).

A)

MDR	Nº of Isolates
B+A+T	23
B+A+S+T	9
B+C+A+T	6
B+C+A+S+T	6
F+A+T	5
C+A+S	3
B+C+F+A+T	3
B+A+S	3
C+F+A+T	2
B+C+A+S	2
A+S+T	2
F+A+S+T	1
C+A+S+T	1
C +A+S+T	1
B+C+T	1
B+C+S+T	1
B+C+F+T	1
B+C+F+A+S+T	1
Total	71

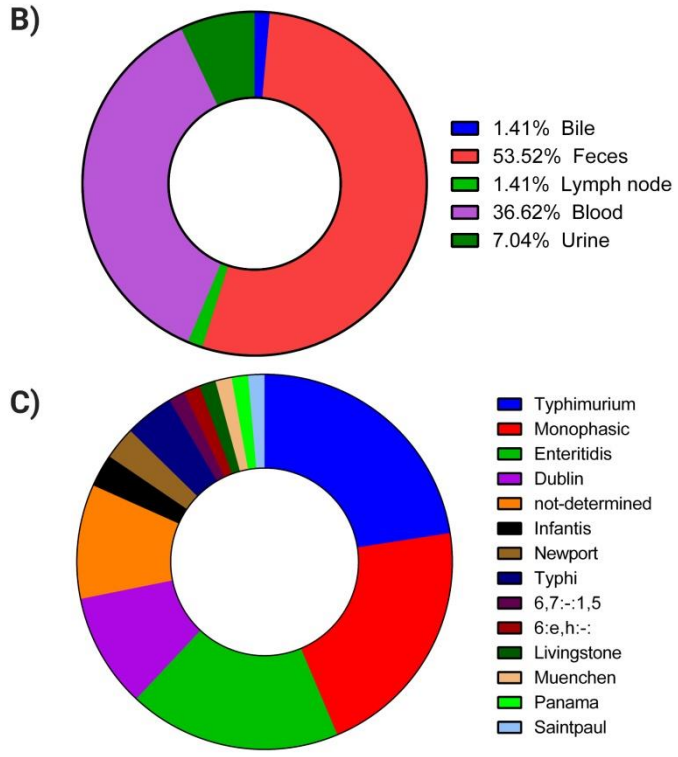


Figure 01. Multidrug-resistant (MDR) isolates from the present study according to the Kirby-Bauer test. A) MDR patterns found and the corresponding number of isolates. A: Aminoglycosides; S: sulfonamides and trimethoprim; T: tetracycline; B: Beta-lactams; F: Fluoroquinolones; C: Chloramphenicol. B) The percentage of MDR isolate's sources. C) The percentage of MDR isolates serovars.

5.1.2. ESBLs Phenotypic Detection

Samples considered resistant against carbapenems or cephalosporins were selected to double-disc synergy test aiming the detection of ESBL (Extended Spectrum Beta-Lactamases) enzymes correlated with second and third-generation cephalosporins or carbapenem resistance. Therefore, samples presenting resistance against amoxicillin were not selected.

Among the isolates tested 11 samples were positive for the production of ESBLs enzymes; Table 07 shows the information about each of them. Using inhibitors of carbapenemase type of enzymes, none of the samples were positive. Among the ESBL-producing samples, six were multi-resistant and one of them isolated from blood. Three samples resistant to cefoxitin (a second-generation cephalosporin) were inhibited by cloxacillin, a profile correlated with the production of *ampC* type of enzymes. The other samples resistant to third-generation cephalosporins were inhibited by clavulanic acid, which

could correspond to different varieties of ESBL enzymes making necessary further molecular analysis to classify the type of ESBL produced by them.

Table 07. Description of ESBL-producer isolates according to double disk synergy test results.

ID/Year	Source	Serovar	Inhibitor*	Resistance Phenotype**
38/2000	Feces	Enteritidis	CLA	CAZ/CTX
2/2006	Feces	Enteritidis	CLA	AML/CTX
1/2007	Blood	1,4,5,12:i:-	CLO	AML/FOX/S/SXT/TE
28/2007	Feces	Typhimurium	CLO	AML/CT/C/FOX/ENR/CN/S/TE
1/2007	Blood	Dublin	CLA	CTX/CAZ/S
520/2008	Feces	Enteritidis	CLA	CTX/CAZ/IPM/CN/S
1061/2009	Feces	Typhimurium	CLA	AML/CTX/C/S/TE
1139/2009	Feces	Typhimurium	CLA	AML/CTX/C/S/TE
1/2012	Blood	1,4,5,12:i:-	CLO	FOX/TE
709/2015	Feces	Muenchen	CLA	AML/CTX/CAZ/CN/S/TE
382/2017	Blood	S. spp	CLA	AML/CTX

*CLA:clavulanic acid; CLO: cloxacillin **CAZ: ceftazidime; CTX: cefotaxime; AML: amoxicillin; FOX: cefoxitin; S: streptomycin; SXT: sulfamethoxazole+ trimethoprim; TE: tetracycline; CT: colistin; C: chloramphenicol; ENR: enrofloxacine; CN: gentamycin; IPM: imipenem.

5.1.3. Minimal Inhibitory Concentration (MIC)

As previously described, samples considered resistant to colistin in the Kirby-Bauer test were selected for MIC testing. Among 810 tested in the Kirby-Bauer test, 17 were considered possibly resistant. MIC was performed following CLSI M07-A10 (2015) parameters, and samples were considered resistant when MIC results were ≥2 μg/ml. Only one sample was considered resistant presenting a MIC of 8 μg/ml. The isolate was a monophasic variant isolated from feces, also resistant to aminoglycosides.

5.2.Whole-genome sequencing

According to the resistance profile and epidemiological importance of the isolates, we selected ten isolates to whole-genome sequencing (WGS). Table 08 shows the information of each isolate selected for WGS according to their source, taxonomy, resistance phenotype, and MLST. The serovar and subspecies shown below were confirmed with WGS data using SeqSero and KmerFinder.

Table 08. Information and MLST results of each isolate submitted to whole-genome sequencing.

ID	Source	Subspecies	Serovar	Phenotype*	MLST
520/08	Feces	*enterica*	Enteritidis	CTX/CAZ/MEM/CN/S	ST11
14/05	Feces	*enterica*	Infantis	AML/S/SXT/TE	ST32
725/16	Feces	*enterica*	Monophasic	AML/CIP/ENR/C/CN/S/ SXT/TE	ST19
303/06	Blood	*enterica*	Typhi	S	ST2
328/06	Blood	*enterica*	Typhi	S	ST2
385/06	Blood	*enterica*	Typhi	S	ST2
13/11	Blood	*enterica*	Typhi	AML/S/SXT	ST1
30/10	Urine	*diarizonae*	61:c:z35	S	ST1845
08/16	Blood	*diarizonae*	61:-:-	S	ST1845
644/10	Feces	*salamae*	42:r:-	S	ST1208

* Resistance phenotype: CTX: cefotaxime; CAZ: ceftazidime; MEM: meropenem; CN: gentamycin; S: streptomycin; AML: amoxicillin; SXT: sulfamethoxazole+ trimethoprim; TE: tetracycline; CIP: ciprofloxacin; ENR: enrofloxacine; C: chloramphenicol.

Additionally, the isolate 725/16 was confirmed as a Typhimurium monophasic variant with the alignment of the operon *fljBA* from the reference strain *S.* Typhimurium LT2. According to the results shown in Figure 02 our isolate presented deletions at *fljA*, the promoter *hin,* and the regions STM2762, STM2763, STM2766, and STM2767 (represented as green arrows). Therefore, our isolate only expresses the flagellin *fliC* and there is no phase variation likewise Typhimurium, which confirms it as a monophasic variant isolate.

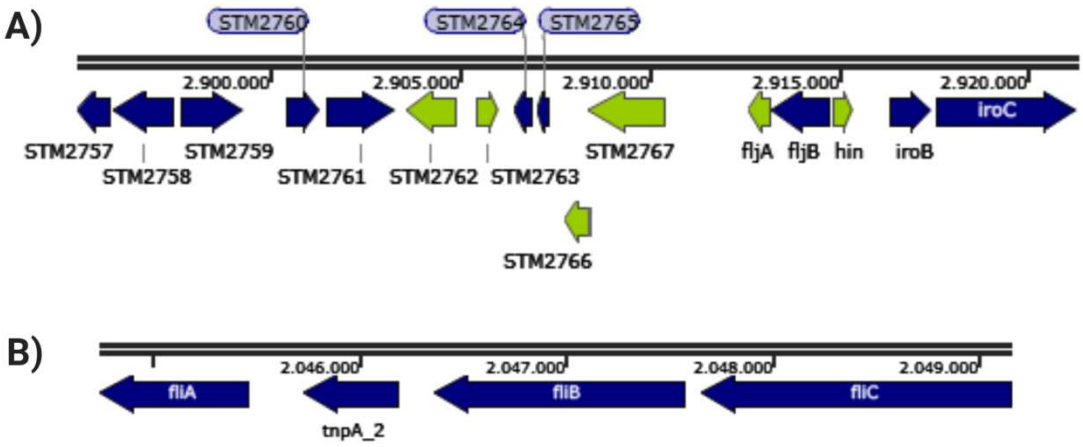


Figure 02. Schematic representation of the operon *fljBA* (A) and the *fliC* region (B) of the monophasic variant isolate 725/16. Blue arrows represent regions that did not suffer deletions, whereas green arrows show regions that were deleted when compared with Typhimurium LT2.

5.2.1. Antimicrobial Resistance Genotype

Antimicrobial resistance factors were analyzed using ResFinder and CARD with default parameters of ≥ 95% protein identity. Table 09 presents factors that were common to all isolates sequenced, except for the gene *aac(6')-ly* (aminoglycoside resistance gene) that was not detected only in the monophasic variant isolate 725/16. All isolates harbored genes encoding for efflux pumps (*mdtK*, *msbA*, *emrB*), regulatory genes responsible for modulating efflux pumps expression (*crp, sdiA, baeR, marA,* and *hns*), and mutations at genes correlated with fosfomycin resistance (*glpT* and *uhpT*), elfamycin resistance (*eftu*), and peptides resistance (*bacA*). Moreover, the isolates presented a mutation correlated with the overexpression of *soxRS,* a superoxide and nitric oxide sensing system that regulates the expression of efflux pumps. These factors were found even in isolates that were only resistant to streptomycin, such as the *S. diarizonae, S. salamae,* and Typhi (isolated in 2006) isolates.

Table 09. Description of antimicrobial resistance factors and their corresponding antimicrobial class and resistance mechanism presented by all isolates herein sequenced.

Genes	Drug Classes	Resistance Mechanisms
eftu[1]	Elfamycin	Antibiotic target alteration
sdiA	Fluoroquinolone, cephalosporin, glycylcycline, penam, tetracycline, rifamycin, phenicol, triclosan	Antibiotic efflux modulation
mdtK	Fluoroquinolone	Antibiotic efflux
crp	Macrolide, fluoroquinolone, penam	Antibiotic efflux modulation
bacA[1]	Peptide antibiotic	Antibiotic target alteration
glpT[1]	Fosfomycin	Antibiotic target alteration
baeR	Aminoglycoside, aminocoumarin	Antibiotic efflux modulation
msbA	Nitroimidazole antibiotic	Antibiotic efflux
emrB	Fluoroquinolone	Antibiotic efflux
uhpT[1]	Fosfomycin	Antibiotic target alteration
soxRS[2]	Fluoroquinolone, monobactam, carbapenem, cephalosporin, glycylcycline, cephamycin, penam, tetracycline, rifamycin antibiotic, phenicol, triclosan	Antibiotic target alteration, antibiotic efflux modulation, reduced permeability to antibiotic
marA	Fluoroquinolone, monobactam, carbapenem, cephalosporin, glycylcycline, cephamycin, penam, tetracycline, rifamycin, phenicol, triclosan, penem	Antibiotic efflux modulation, reduced permeability to antibiotic

hns	Macrolide, fluoroquinolone, cephalosporin, cephamycin, penam, tetracycline	Antibiotic efflux modulation
aac(6')-Iy[3]	Aminoglycoside	Antibiotic inactivation

[1]protein variant; [2]protein overexpression; [3]absent only in the monophasic variant 725/16

The multidrug-resistant isolates analyzed, presented additional factors associated with antimicrobial resistance (Table 10). The Enteritidis isolate 520/08, the only positive for ESBL production in the double-disc synergy test, did not present any genes associated with ESBL enzymes at ≥ 95% identity. Nevertheless, this same isolate presented 91% identity with the gene *ampH* that encodes a chromosomal class C beta-lactamase.

The presence of this same gene *ampH* was then investigated in all samples. Likewise the isolate 520/08 all the other samples presented the gene *ampH* with 91% identity, even though the other isolates did not present the phenotype associated with ESBL production. The CARD software used to detect the antimicrobial resistance genes compared our samples with the *ampH* gene sequence of *E. coli.* Hence, we used *Salmonella* Typhimurium LT2 *ampH* sequence to align with our samples and detect possible mutations at this locus using BLASTn.

The alignment results of each sample are shown in Appendix 04. According to the results the monophasic variant was the only isolate to not present any mutations compared to the LT2 strain. Both *S. diarizonae* isolates presented 96% identity with the same 45 SNPs in the gene and its promoter, while *S. salamae* isolate presented 97% identity and 30 SNPs. All Typhi isolates from 2006 presented the same 13 SNPs in this region, while the Typhi 13/11 presented 12 SNPs in addition to those 13 variations found in the other Typhi. The Infantis isolate presented 15 SNPs, and 5 of them were also found in the Enteritidis isolate. In total, the Enteritidis 520/08 possessed only 9 SNPs, presenting the smallest number of SNPs found, and none of them located in the promoter region. Considering that a natural variation of this locus among different serovars seems to occur naturally, further investigations using RT-PCR or a transcriptome analysis are needed to understand rather specific SNPs could lead to an overexpression of this gene resulting in beta-lactam resistance.

Some of the MDR isolates shared the same antimicrobial resistance factors. Mutations in the gene *gyrA,* associated with fluoroquinolone resistance, were found in the Enteritidis 520/08, Typhi 13/11, and monophasic variant 725/16 isolates. Only the isolate 725/16 presented the resistance phenotype against fluoroquinolones. Another resistance factor found in more than one isolate was the gene *golS*, associated with beta-lactam resistance, presented by the Infantis 14/05, monophasic variant 725/16, and the Enteritidis 520/08 isolates. Another

feature related to beta-lactam resistance presented by more than one isolate was the gene *bla*$_{tem-1}$. This resistance gene was found in the isolates Typhi 13/11 and monophasic variant 725/16, both resistant to amoxicillin.

The monophasic variant 725/16 presented the widest variety of factors associated with antimicrobial resistance, all factors corresponding to its phenotype (Table 08). Besides the *gyrA* variation, already mentioned, the sample 725/16 was the only one to present another gene correlated with fluoroquinolone resistance, the *qnrE1* gene. This gene is a plasmid-mediated quinolone resistance gene (PMQR) that has been recently described for the first time in Argentina (Abornoz et al., 2017). Comparing the context surrounding this gene to the originally described one in *K. pneumoniae* Q1130, they presented high similarity with the exception of the protein *ahp* that differed in size (Figure 03).

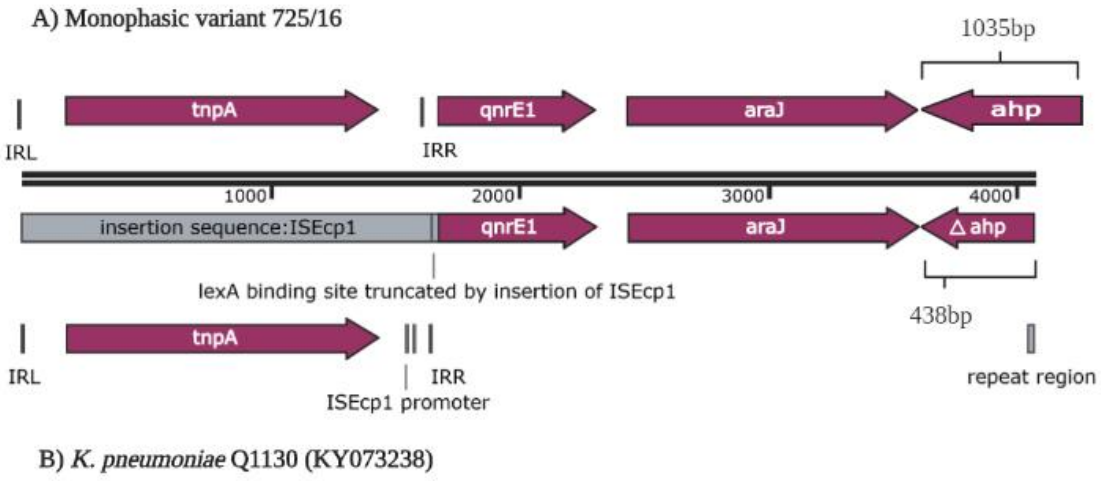


Figure 03. Comparison between the genetic context surrounding *qnrE1* gene of the A) Monophasic variant 725/16 from this study and the B) *K. pneumoniae* Q1130 (accession number KY073238). Created with Biorender.com.

Regarding beta-lactam resistance, besides genes *bla*$_{tem-1}$ and *golS*, the isolate 725/16 also presented the efflux systems genes *mdsAB*. Among the beta-lactams herein tested the isolate was only resistant to amoxicillin, but considering the efflux pumps found it shows the potential of being resistant to other beta-lactams not tested in this study. Moreover, this sample presented two genes related to aminoglycoside resistance (genes *aadA1* and *aac(6')-Iaa*) that were not found in any other samples. This isolate also presented genes *sul1*, *dfrA1*,

tetA, and *floR* which are compatible with its resistance phenotype against sulfamethoxazole, trimethoprim, tetracycline, and chloramphenicol respectively.

The isolate Typhi 13/11 presented not only the genes *gyrA* and bla_{tem-1} already mentioned, but also other genes correlated to its resistance phenotype. This sample was the only one to present genes *aph(6)-Id* and *aph(3'')-Ib*, both associated with aminoglycoside resistance. Moreover, the isolate presented *dfrA14* and *sul2* genes associated with trimethoprim and sulfamethoxazole resistance respectively.

Table 10. Presence (+) of genetic factors associated with antimicrobial resistance in multidrug-resistant isolates according to WGS data.

Genes	**Drug Class**	**Enteritidis**	**Infantis**	**Monophasic**	**Typhi**
		ID520/08	**ID14/05**	**ID725/16**	**ID13/11**
golS	Monobactam, carbapenem, cephalosporin, cephamycin, penam, phenicol, penem	+	+	+	
mdsAB	Monobactam, carbapenem, cephalosporin, cephamycin, penam, phenicol, penem			+	
bla_{tem-1}	Monobactam, cephalosporin, penam, penem			+	+
aph(6)-Id	Aminoglycoside				+
aph(3'')-Ib	Aminoglycoside				+
aadA1	Aminoglycoside			+	
aac(6')-Iaa	Aminoglycoside antibiotic			+	
gyrA[1]	Fluoroquinolone	+		+	+
qnrE1	Fluoroquinolone			+	
dfrA1	Diaminopyrimidine			+	
dfrA14	Diaminopyrimidine				+
sul1	Sulfonamide			+	
sul2	Sulfonamide				+
floR	Phenicol			+	
tetA	Tetracycline			+	

1: Protein variant

5.2.2. Presence of Mobile Elements

The presence of bacteriophages and plasmids were also evaluated and are described in Table 11. Only the MDR isolates Typhi 13/11, Enteritidis 520/08, and the monophasic variant 725/16 presented plasmids according to PlasmidFinder. The Enteritidis 520/08 presented

plasmids from two incompatibility groups IncFII and IncFIB, the last one also being present in Typhi 13/11 isolate. Moreover, three different plasmid replicon types (IncFIA, IncHI2 and IncHI2A) were detected in the monophasic variant isolate 725/16.

Regarding the presence of bacteriophages, all isolates presented at least one intact sequence as shown in Table 11. The monophasic variant and the *S. salamae* isolates carried sequences from the lambdoid phage Gifsy-1, while the Enteritidis carried Gifsy-2, a phage from the same group. Using BLASTn to detect virulence factors encoded by Gifsy phages the *sodC-1* gene encoded by Gifsy-2 was found in Enteritidis 520/08 and in the monophasic variant 725/16, which did not harbor the intact sequence of Gifsy-2, but it presented its partial sequence including the *sodC-1* gene. In order to confirm that isolates 725/16 and 520/08 presented the *sodC-1* encoded by Gifsy-2 not the chromosomal copy of this gene, BLASTn was used to compare the prophage protein *sodC-1* found in *S.* Typhimurium LT2 with both samples, which presented 100% of identity confirming the result. The presence of genes *gogB, gogA,* and *gipA,* virulence factors encoded by Gifsy-1 phage, were also investigated using BLASTn. The only gene present in our samples was *gogB* found in the 725/16 isolate.

Another lambda-like group found was the phage SEN34 detected in all Typhi isolates and the *S. diarizonae* 08/16. Moreover, most of our samples presented phages from the P-2 group, such as Sal-3, L-413C, Fels2, and HP2. The Enteritidis isolate showed the presence of Sal-3, also found in the two *S. diarizonae* isolates and *S. salamae*. The Fels2 phage was detected in Typhi isolates 13/11, 303/06, and 328/06. The Infantis isolate was the only one harboring the *Yersinia* spp. phage L-413C, while just the *S. salamae* 644/10 harbored the HP2 phage.

Table 11. Detection of plasmids and prophage intact sequences in each isolate.

ID	Serovar	Plasmids	Phages
520/08	Enteritidis	IncFIB/IncFII	Sal3[a]/Gifsy-2[b]
14/05	Infantis	-	*Yersinia* L-413C[a]
725/16	Monophasic	IncFIA/IncHI2/IncHI2A	Gifsy-1[b]
303/06	Typhi	-	Fels2[a]/SEN34[b]
328/06	Typhi	-	Fels2[a]/SEN34[b]
385/06	Typhi	-	SEN34[b]
13/11	Typhi	IncFIB	Fels2[a]/SEN34[b]

30/10	*diarizonae*	-	Sal3[a]
08/16	*diarizonae*	-	SEN34[b] / Sal3[a]
644/10	*salamae*	-	HP2[a] / Gifsy-1[b]/ Sal3[a]

[a]P2-like prophages; [b] Lambda group

5.2.3. Virulence Factors and *Salmonella* Pathogenicity Islands

According to SPI-Finder results, our samples presented different intact sequences (≥95% identity) of *Salmonella* Pathogenicity Islands (SPIs) as shown in Table 12. Moreover, BLASTn and MAUVE were used to confirm the presence of the six mainly SPIs (SPI-1 to SPI-6) because our sequences are draft genomes, hence SPI-Finder was not accurate detecting SPI regions that were separated into different contigs after assembly. Considering that, the core genome of the SPI-1 to SPI-6 from the reference strain Typhimurium LT2 was used based on the same regions chosen by the software SPI-Finder, and they were aligned against the isolates of this present study. Figures 04-09 show the final results of the alignments with a schematic representation of each SPI (SPI-1 to SPI-6) from the reference strain at the top, and the colored lines named with letters below each SPI represent the deletion patterns (blue lines) and conserved regions (red lines) found among the samples

Table 12. Distribution of intact (>95% identity) *Salmonella* Pathogenicity Islands (SPIs) among *Salmonella* spp. isolates.

ID	Serovar/Subspecies	SPIs
520/08	Enteritidis	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-8/SPI-9/SPI-10
14/05	Infantis	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-13/SPI-14/ C63PI[a]
725/16	Monophasic	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-13/SPI-14/ C63PI[a]
303/06	Typhi	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-8/SPI-9/SPI-10
328/06	Typhi	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-8/SPI-9/SPI-10
385/06	Typhi	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-8/SPI-9/SPI-10
13/11	Typhi	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-6/SPI-8/SPI-9/SPI-10
30/10	*diarizonae*	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-13/C63PI[a]
08/16	*diarizonae*	SPI-1/SPI-2/SPI-3/SPI-4/SPI-5/SPI-13/C63PI[a]
644/10	*salamae*	SPI-1/SPI-2/SPI-3/SPI-13/C63PI[a]

[a] C63PI: Centisome 63 Pathogenicity Island

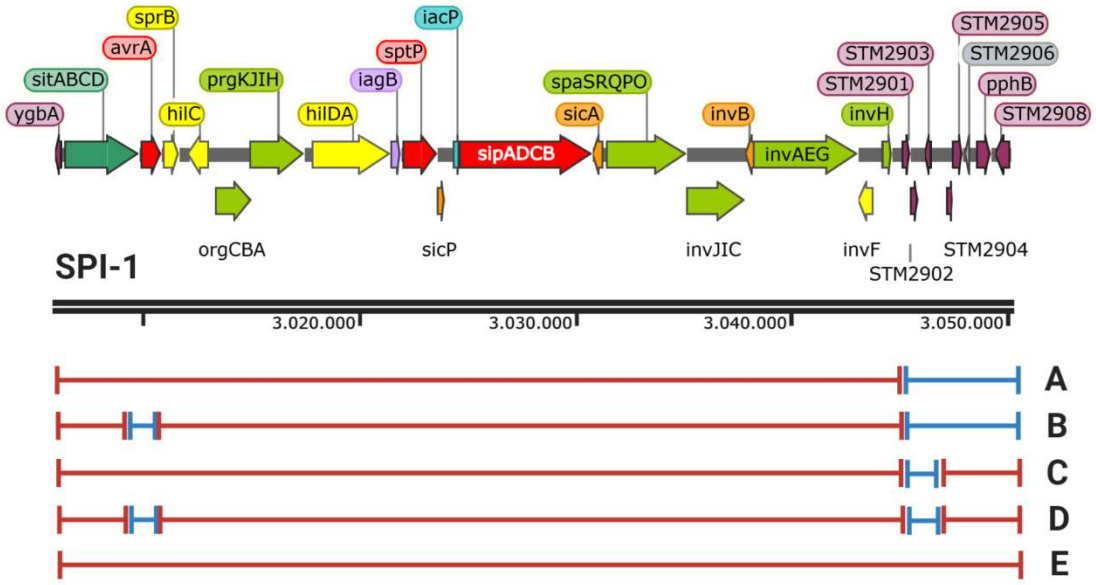


Figure 04. Schematic representation of SPI-1 of *S.* Typhimurium LT2. Blue lines represent deletions among samples herein studied and red lines represent intact regions. A) *S. diarizonae* 30/10 and 08/16. B) *S. salamae* 644/10. C) *S.* Enteritidis 520/08. D) *S.* Typhi: 13/11, 303/06, 328/06, 385/06. E) *S.* Infantis 14/05 and monophasic variant 725/16.

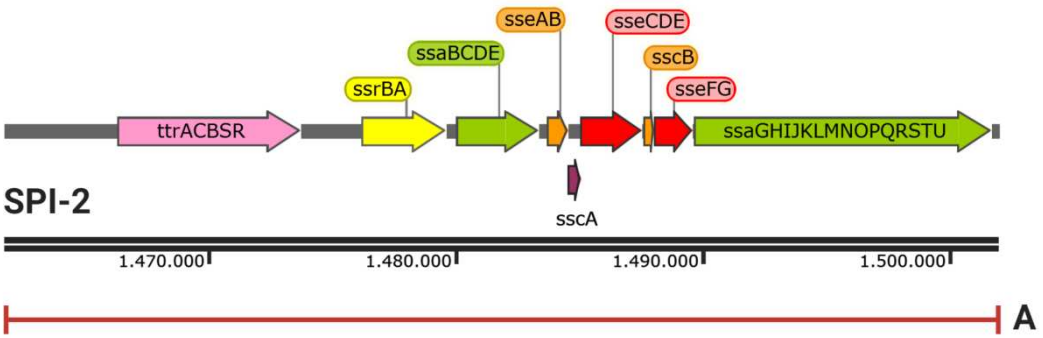


Figure 05. Schematic representation of SPI-2 of *S.* Typhimurium LT2. Red lines represent intact regions presented by samples herein studied. A) All isolates herein studied.

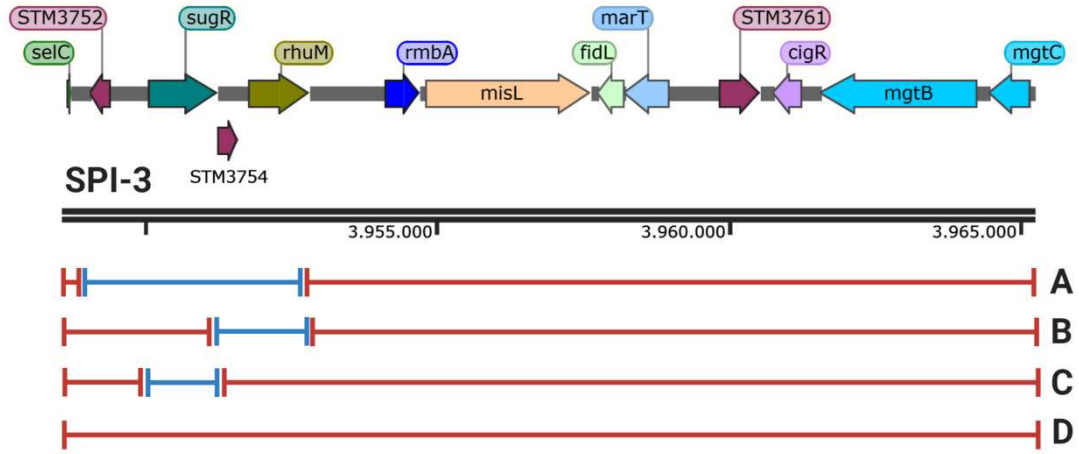


Figure 06. Schematic representation of SPI-3 of *S*. Typhimurium LT2. Blue lines represent deletions among samples herein studied and red lines represent intact regions. A) *S.* diarizonae 30/10 and 08/16, *S.* Infantis 14/05. B) *S*. salamae 644/10. C) *S*. Typhi: 13/11, 303/06, 328/06, 385/06. D) *S*. Enteritidis 520/08 and monophasic variant 725/16.

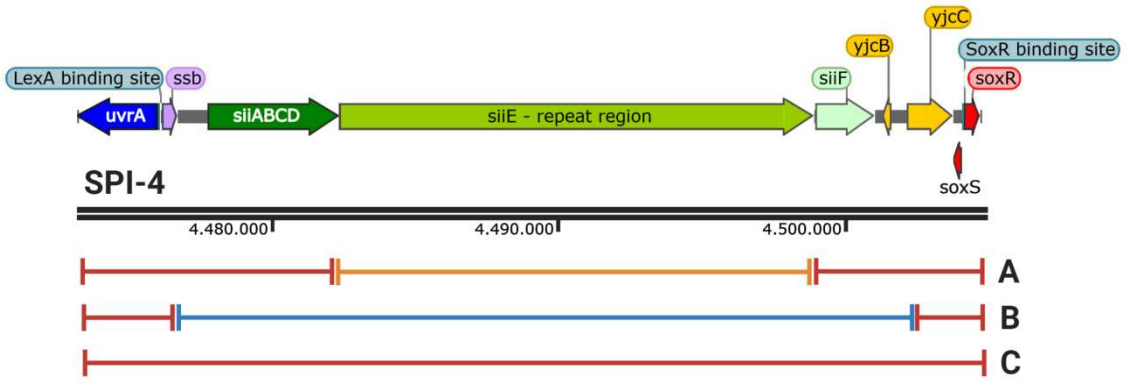


Figure 07. Schematic representation of SPI-4 of *S*. Typhimurium LT2. Blue lines represent deletions among samples herein studied and red lines represent intact regions. A) *S.* diarizonae 30/10 and 08/16. B) *S.* salamae 644/10. C) *S.* Typhi: 13/11, 303/06, 328/06, 385/06, *S*. Enteritidis 520/08, *S*. Infantis 14/05, and monophasic variant 725/16.

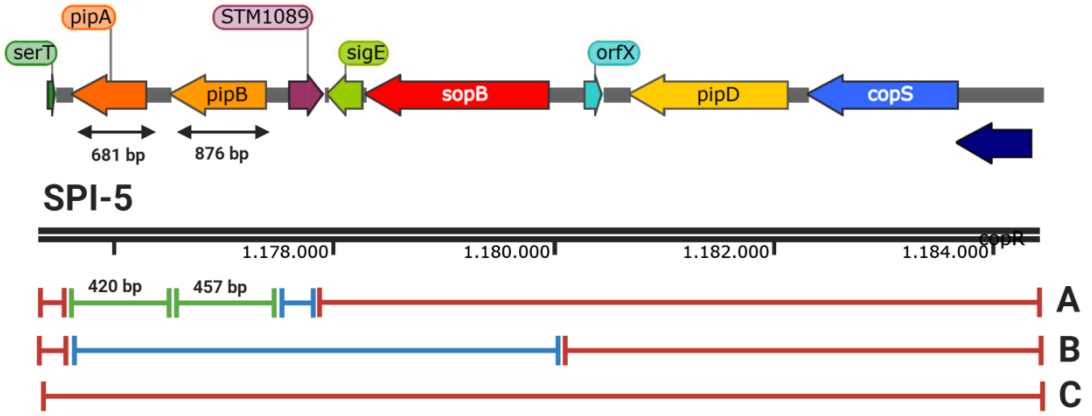


Figure 08. Schematic representation of SPI-5 of *S.* Typhimurium LT2. Blue lines represent deletions among samples herein studied and red lines represent intact regions. A) *S.* diarizonae 30/10 and 08/16. B) *S.* salamae 644/10. C) *S.* Typhi: 13/11, 303/06, 328/06, 385/06, *S.* Enteritidis 520/08, *S.* Infantis 14/05, and monophasic variant 725/16.

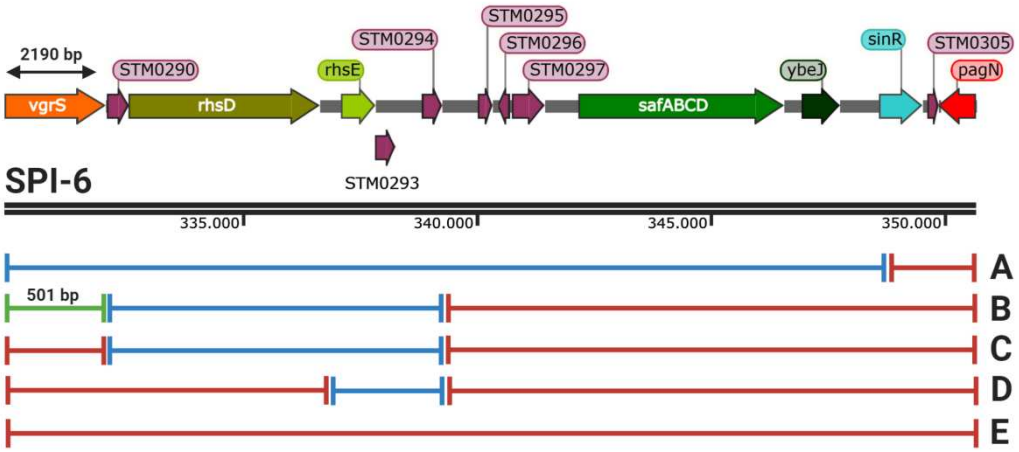


Figure 09. Schematic representation of SPI-6 of *S.* Typhimurium LT2. Blue lines represent deletions among samples herein studied and red lines represent intact regions. A) *S.* diarizonae 30/10 and 08/16, *S.* salamae 644/10. B) *S.* Enteritidis 520/08. C) *S.* Infantis 14/05. D) *S.* Typhi: 13/11, 303/06, 328/06, 385/06. E) Monophasic variant 725/16.

All isolates herein studied (the non-*enterica* and *enterica* subspecies) presented 100% identity of the LT2 SPI-2 sequence (Figure 05), while SPI-1 and SPI-3 were present, but with small deletions among some samples (Figure 04 and Figure 06). The *S. diarizonae* isolates and the typhoidal isolates lacked the *avrA* gene from SPI-1, also the non-*enterica* samples presented deletions within hypothetical proteins annotated as STM2901-2905/2908 and gene

pphB (Figure 04). Within the SPI-3 region, the *S. diarizonae* isolates and the Infantis isolate presented deletions at genes *sugR*, *rhuM,* and regions representing hypothetical proteins. The *S. salamae* isolate only presented deletions at *rhuM* and STM3754 (hyphotetical protein), and all typhoidal isolates lacked *sugR* (Figure 06). All *enterica* subspecies isolates presented the intact SPI-4 and SPI-5.

All *enterica* subspecies isolates presented the intact SPI-4, whereas non-*enterica* samples presented deletions. The isolate *S. salamae* was the only one to lack almost the entire SPI-4 presenting only the *uvrA, yjcBC*, and *soxRS* (Figure 07). The *S. diarizonae* isolates *siiE* region presented a low-quality alignment to the same gene in LT2 (Figure 07). Only part of the gene aligned, but *siiE* is a 16680 bp repeat region, hence it is a challenging region to assembly correctly. Therefore, this gene might not be deleted, but it is a limitation of using a draft genome.

Likewise SPI-4, the SPI-5 of non-*enterica* isolates lacked some regions. The *S. salamae* SPI-5 was the only one to present low identity (50%) when compared to LT2, lacking the genes *pipA, pipB, sigE,* and *sopB.* The *diarizonae* isolates lacked STM1089, and presented smaller *pipA* and *pipB* compared to LT2 (Figure 08).

The SPI-6 was only present among *enterica* subspecies within our isolates, but they presented minor deletions that differed between samples (Figure 09). The monophasic variant 725/16 did not harbor any deletions, while all typhoidal isolates lacked STM0293/0294, and a gene homologous to *rhsE* of *E. coli*. The Enteritidis 520/08 and the Infantis 14/05 lacked STM0290/0293/0294, and the region homologous to *rhsDE* in *E.coli*. Additionally, the Enteritidis 520/08 was the only *enterica* isolate to present a partial deletion of the gene homologous to the *vgrG* in *Pseudomonas* spp., annotated as *vgrS* in the LT2, while the LT2 gene is 2190 bp the 520/08 gene presented a 501 bp size.

The *S. diarizonae*, Infantis, and the monophasic variant isolates were the only ones to present the SPI-13. Furthermore, they presented the centisome C63PI also detected in the *S. salamae* sample. Concerning the Typhi isolates, all samples showed the same SPIs, and they shared the same results as the Enteritidis isolate, whereas the monophasic variant and the Infantis presented the same islands.

Besides the SPIs, the presence of other virulence factors was also investigated using the VFDB database, and all the results can be found in Appendix 05-07. All samples were

compared to the reference strain *Salmonella* Typhimurium LT2. Additionally, in Appendix 5, where it is shown the Enteritidis, Infantis and monophasic variant results, the reference strain *Salmonella* Enteritidis P125109 was added for comparison. The reference strain *Salmonella* Typhi TY2 was added to Appendix 6 as a comparison to the other *Salmonella* Typhi samples, and the reference strain *Salmonella arizonae* RSK2980 was added to Appendix 7 with the *S. diarizonae* and *S. salamae* virulence factors results.

Regarding the subspecies *enterica* samples (Appendix 05/06), all isolates presented divergences related to the presence of adherence genes and secretion system proteins from the SPI-1 and SPI-2. However, important T3SS genes and other virulence factors from SPI-1/2/3/4/5, such as *invA-J, phoPQ, sptP, sipABC, sopE, sifAB, ssaCDE, sseFG, ssrA, sscAB, mgtBC, sopB, pipB* and *pipA,* were present in all samples including typhoidal isolates. The fimbrial gene cluster *lpf* was present in all non-typhoidal subspecies *enterica* isolates. Another important fimbrial gene cluster found among all subspecies *enterica* isolates was the *saf* cluster, located in SPI-6.

The Enteritidis 520/08 isolate was the only one that presented genes from the locus *spvABCDR*, *pefABCD,* and *rck,* which are all harbored by the virulence plasmid IncFII+IncFIB, known as the pSEV, harbored by this sample. The prophage-encoded gene *sodC1* was only present in the Enteritidis and monophasic variant as mentioned before. The monophasic variant 725/16 was the only one presenting the genes *gogB* and *gtrA*, the last one associated with immune system evasion. This same isolate also presented the invasin A from *Yersinia* spp., also detected in the Typhi 13/11 isolate. Another virulence factor not commonly found among the isolates, but detected in the monophasic variant 725/16 and *S. salamae* 644/10 was the gene *ibeB*. This gene encodes a protein associated with the invasion of brain endothelial cells.

Comparing the virulence factors presented by the Typhi isolates (Appendix 06) the samples 303/06 and 328/06 presented the same genes. Even though all Typhi isolates from 2006 belong to the same sequence type, the isolate 328/06 presented minor divergences among adherence genes compared to the other Typhi isolates from the same outbreak. Overall, the three 2006 isolates presented most of the capsular and adherence genes, besides genes from the SPI-1 and SPI-2 found in the reference strain Typhi TY2.

Although the subspecies non-*enterica* presented some deletions among SPI-1, SPI-3, and SPI-5 in their genome, they did present several virulence factors from these islands that

were also found in the subspecies *enterica* isolates, such as *invA, sipABC, phoPQ, ssra, sseFG, sscAB, sopE,* and *mgtBC,* besides presenting an intact SPI-2 (Appendix 07). Additionally, all three non-*enterica* isolates harbored the gene *cdtB,* also found in all of our *S.* Typhi isolates. This gene is correlated with the production of a toxin in *Salmonella* spp.

In addition to the virulence factors presented by all samples, other genes correlated with bacterial pathogenicity were only present in the *S. salamae* 644/10 isolate, such as the LEE locus (*locus of enterocyte effacement*), the gene *usp,* and ACE-like genes, all virulence factors from pathogenic *E. coli*. However, 644/10 was the only isolate to not present *pipA*, *pipB* and *sopB*, which are effector proteins necessary during the infection process.

Both *S. diarizonae* isolates 08/16 and 30/10 shared unique virulence genes such as Type VI secretion system proteins from *E. coli* and *Klebsiella* spp, yersiniabactin genes, and *pilW* genes both from *Yersinia* spp. The isolate 08/16 was the only one to present *escS,* a gene from the LEE locus, while the *pilQRS* operon, which is a type IV pili from *Yersinia* spp., was only found in the isolate 30/10. Additionally, the *S. diarizonae* isolates presented partial deletions of genes *pípA* and *pipB* as already mentioned.

5.3.*Galleria mellonella in-vivo* Tests

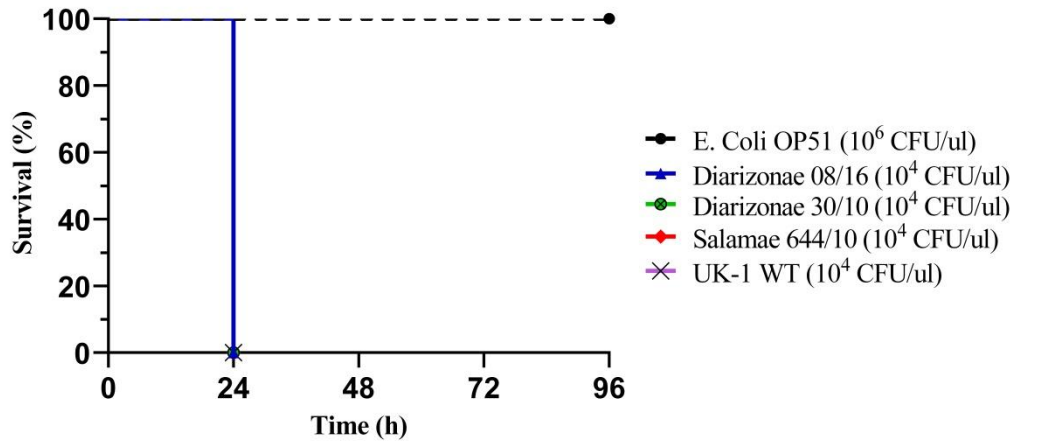
The *Galleria mellonella* insect model was chosen for the *in-vivo* assays using the non-*enterica* subspecies isolates of this study. The *Escherichia coli* OP50-1 was used as a non-virulent control at concentrations of 10^6 and 10^5 CFU/µl. During the 96h observation period 100% of larvae inoculated with *E. coli* OP50-1 survived. Phosphate-buffered saline (PBS) (10 mM) was also inoculated as a negative control and did not kill any larvae during the assay. The reference strain *Salmonella Typhimurium* UK-1 was used as our virulent-control and it was inoculated at the same concentrations the non-*enterica* samples 10^4, 10^3 and 10^2 CFU /µl.

As shown in Figure 10-A, using the inoculum concentration of 10^4 CFU/µl, the survival rate was 0% to all non-*enterica* samples and the virulent control UK-1, all larvae died within 24h. Whereas inoculating 10^3 CFU/µl of the bacterial isolates, 100% of larvae inoculated with the virulent control UK-1 survived. Nevertheless, using this same concentration (10^3 CFU/µl) all larvae inoculated with the non-e*nterica* isolates were killed after 24h (Figure 10-B).

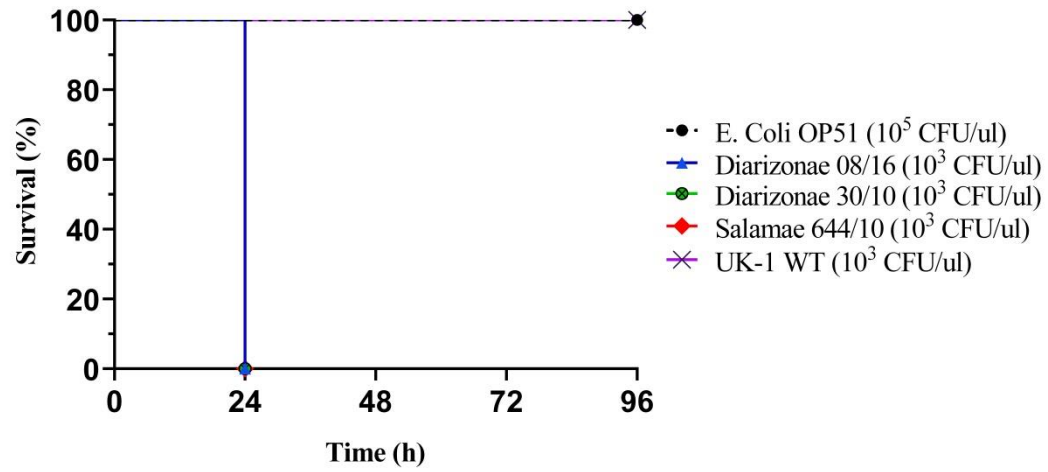
Injecting 10^2 CFU/µl of the non-*enterica* samples the survival rates of each isolate diverged (Figure 10-C). The virulent control UK-1 did not kill any larvae within 96h, as well

as the *S. diarizonae* 30/10. In contrast, the *S. diarizonae* 08/16 isolate killed eight larvae (80%) within 24h and presented a 20% survival rate after 96h. The *S. salamae* 644/10 isolate showed a survival rate of 40% after 96h, with five larvae being killed within 24h, and a sixth one killed after 48h.

A)



B)



C)

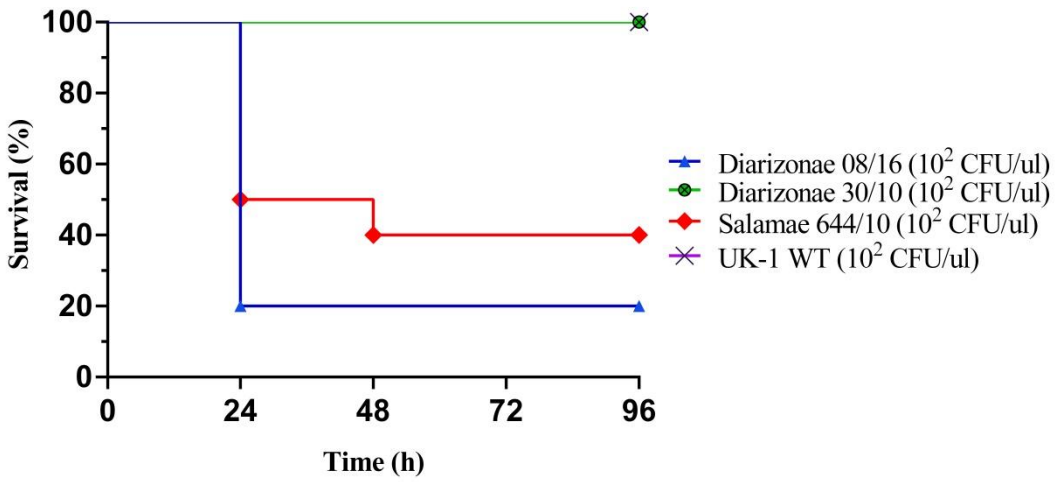


Figure 10. Survival curves in *Galleria mellonella* for *E. coli* OP50-1, *S. diarizonae* 08/16 and 30/10, *S. salamae* 644/10, and *S.* Typhimurium UK-1 isolates during 96h using 10 larvae for each bacterial isolate. A) *E. coli* OP50-1 inoculum concentration: 10^6 CFU/µl. All other isolates: 10^4 CFU/µl. B) *E. coli* OP50-1 inoculum concentration: 10^5 CFU/µl. All other isolates: 10^3 CFU/µl. C) Inoculum concentrations of isolates: 10^2 CFU/µl. These are the representative results of three independent experiments.

5.4. Macrophage Invasion and Survival Assay

Both *S. diarizonae* and the *S. salamae* isolates were able to invade and survive the J774 macrophages as shown in Figure 11. Using the two-way analysis of variance (ANOVA) there were no significant differences among these samples when compared to the reference strain *S.* Typhimurium 14028. Moreover, the number of bacterial cells recovered in 2h and 6h for each sample was statistically similar. Therefore, none of the samples, including the reference strain, were able to increase in number intracellularly during the period of 6h.

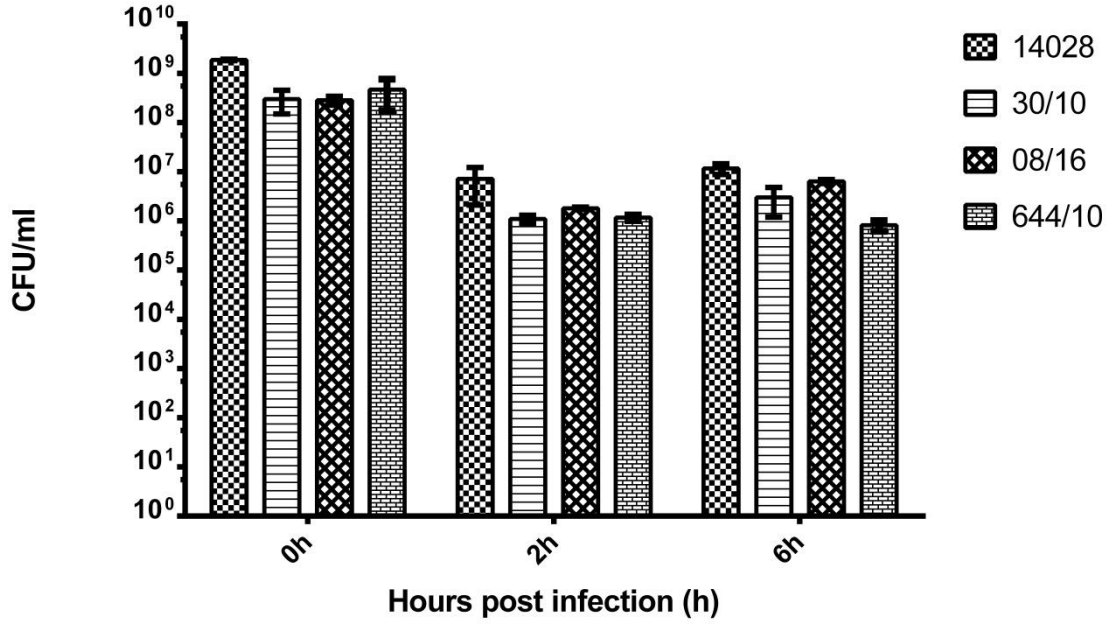


Figure 11. Invasion and surveillance of *Salmonella diarizonae* (30/10 and 08/16), *Salmonella salamae* (644/10), and *Salmonella Typhimurium* ATCC 14028 isolates in J774 macrophages.

6. Discussion

6.1.Antimicrobial Resistance Phenotype

Among the 810 samples tested, the drugs that presented the highest percentage of resistant samples were streptomycin (30.12%), tetracycline (13.83%), and amoxicillin (12.72%). These three drugs belong to the aminoglycoside, tetracycline, and penicillin (beta-lactam) antimicrobial classes, respectively. The prevalence of this resistance profile was found among the four most prevalent serovars Enteritidis, Typhimurium, monophasic variant, and Dublin, which together represent 66% of the samples. The typhoid isolates also presented a higher resistance percentage against streptomycin (55.56%) and amoxicillin (16.67%), but the resistance against sulfamethoxazole/trimethoprim was higher (22.22%) compared to the resistance against tetracyclines (5.56%). The three subspecies non-*enterica* isolates only presented resistance against streptomycin.

Resistance against aminoglycosides, tetracyclines, and penicillins has been observed previously in *Salmonella* spp. strains especially from food-producing animals. The Brazilian National Sanitary Surveillance Agency monitored the antimicrobial resistance of *Salmonella*

spp. isolated from poultry and reported a higher percentage of resistance against streptomycin (89.3%), sulfonamides (72.4%), and ampicillin (44.8%) (Brasil, 2008). The prevalence of aminoglycosides and penicillins resistance among *Salmonella* spp. isolated from poultry are similar with the resistance profile herein detected.

Moreover, in a meta-analysis study conducted with *Salmonella* spp. isolated from poultry and humans in Brazil, they reported 46.4% of resistance against sulfonamides, 36.9% against tetracyclines, and 23.6% against ampicillin (a penicillin likewise amoxicillin) in human-origin isolates (Voss-Rech et al., 2017). Even though the studied samples did not show a high resistance percentage against sulfonamides in combination with trimethoprim (6.42%), our results are aligned with the high percentage of resistance against tetracyclines and penicillins.

The prevalence of samples resistant to aminoglycosides, tetracyclines, and penicillins can be correlated to the use of these drug classes in veterinary medicine, especially as growth promoters in food-producing animals. Tetracyclines were used as growth promoters (GP) for decades in Brazil until a normative in 1998 which prohibited the use of tetracyclines and chloramphenicol for therapy purposes in food-producing animals as well as growth promoters (Silva, 2015). Nevertheless, tetracyclines and chloramphenicol residues were still found in food samples after the normative was implemented. Moreover, recent studies have shown that residues of aminoglycosides and penicillins surpassing the limits imposed by Brazilian legislation were also found in food samples with animal origin (Silva, 2015). The sub-therapeutical antimicrobial concentrations of growth promoters and the misuse of antimicrobials in veterinary medicine can select resistant bacteria strains, presenting a threat to human public health through the consumption of food contaminated with resistant bacteria (Brasil, 2008).

Another cause of concern is quinolone/fluoroquinolone-resistant *Salmonella* spp., that has been reported worldwide including in Brazil. For instance, a meta-analysis study in Brazil showed a high number of *Salmonella* spp. isolated from poultry presenting resistance against nalidixic acid (Voss-Rech et al., 2016). Even though our study did not test nalidixic acid, a type of quinolone, our isolates were overall susceptible to fluoroquinolones, with 3.95% resistance against enrofloxacin and 0.37% against ciprofloxacin. Our results differ from international reports showing an increasing number of fluoroquinolone-resistant *Salmonella* spp. in Europe (Veldman et al., 2011), China (Kuang et al., 2018), and the United States

(Karp et al., 2018). Considering fluoroquinolones are the first choice to treat Salmonellosis, a susceptible profile of samples is important to human public health.

Among the resistant samples in this study, 71 (8.77%) were resistant to three or more antimicrobial classes simultaneously and were classified as multidrug-resistant, where 36.66% of them were isolated from blood samples. According to Fisher's exact test the proportion of MDR samples isolated from blood was significantly higher (p=0.0019) when compared to samples isolated from other sources and it presented an odd ratio (OR) of 2.3, meaning samples from blood were two times more likely to be MDR. Additionally, 22.54% (16) MDR samples were Typhimurium and 21.13% (15) were monophasic, which according to Fisher's exact test represents a significant higher proportion (p=0.0005 and p=0.0002 respectively) of MDR samples belonging to these serovars compared to others. The Typhimurium presented a OR=3.2 while the monophasic presented a OR=3.6, thereby among isolates herein studied there were higher odds of monophasic and Typhimurium presenting a MDR phenotype.

The most common MDR pattern herein described (beta-lactam, aminoglycosides, and tetracyclines) have been previously reported in *Salmonella* Infantis isolated from children in Brazilian hospitals (Fonseca et al., 2006). However, a multidrug (MDR) pattern in *Salmonella* spp. that causes great concern for public health is the ACSSuT (ampicillin-amoxicillin, chloramphenicol-florfenicol, streptomycin-spectinomycin, sulfonamides, and tetracycline), but among samples herein tested only six presented such phenotype. Our results do not correspond to previous studies; for instance, a meta-analysis study covering approximately 55 years of research pointed this MDR pattern as the most common one in the United States, especially among Typhimurium, Enteritidis, and Newport serovars (Parisi et al., 2018).

Even though ACSSuT-resistant isolates were not common within our samples three of those isolates came from blood samples, which is a cause of concern. Patients infected by MDR *Salmonella* spp. presented higher odds of hospitalization when compared to patients infected by susceptible-isolates in the United States (Parisi et al., 2018). This highlights the necessity of mitigating MDR isolates dissemination in order to decrease their impact on public health.

Multidrug bacterial infections can be challenging because of the limited options of efficient antimicrobials against them, in cases where MDR *Salmonella* spp. isolates present resistance against fluoroquinolones, cephalosporins and carbapenems are used as a treatment (Basseti et al., 2019). Within the 810 samples herein tested, 11 were considered ESBL-

producers. Even though one Enteritidis (520/08) isolate presented resistance to meropenem, any of the isolates presented the phenotype correlated to KPC-producers, which are inhibited by phenylboronic acid. Three of our isolates were inhibited by cloxacillin, which indicates, according to the functional classification scheme (Bush and Jacoby, 2010), the production of a group-1 cephalosporinase, while eight were inhibited by clavulanic acid, possibly being a group-2 cephalosporinase. Additionally, ten isolates were resistant to more than one beta-lactam, with three being resistant to ceftazidime and cefotaxime and seven being resistant to amoxicillin and at least one cephalosporin, this could indicate the production of more than one enzyme.

Cephalosporinases have been reported in the food industry in different continents worldwide such as Africa (Saravanan et al., 2018), North America (CDC, 2014), South America (Sampaio, 2016), and Europe (Canton et al., 2008). Nevertheless, considering the wide diversity of beta-lactamases, molecular studies are necessary to characterize the genes correlated to the resistance herein described. Further investigations about the ESBL-producer strains can contribute to epidemiological studies on ESBL enzymes dissemination. Thus, we intend in the future to further investigate all ESBL samples from this study through WGS.

When multidrug-resistant bacteria are ESBL-producers and fluoroquinolone resistants, colistin is the last resort antibiotic (Basseti et al., 2019). The only colistin-resistant sample herein found was a monophasic variant isolated from a coproculture, which only presented resistance to streptomycin in addition to colistin. Colistin resistance can be caused by chromosomal mutations or plasmid-mediated genes (*mcr* genes). The dissemination of plasmids harboring this gene among MDR strains can compromise modern medicine due to the lack of efficient antimicrobials to stop the infection. Although our colistin-resistant strain did not present a MDR profile, it possibly harbors the vector responsible for the dissemination of such genotype. Therefore, molecular investigations to understand the mechanisms involved in this resistance are necessary, and this sample will have its genome sequenced in the future.

6.2.Whole-Genome Sequencing

Analyzing the MLST results of samples herein studied the Enteritidis 520/08 was classified as ST11, which is the most prevalent sequence type of this serovar around the world. The ST11 has been previously reported in Brazil from human and non-human sources, however the antimicrobial resistance profile of the strains were not investigated (Campioni et al., 2015).

Among the Typhi isolates, we detected one ST2 (13/11) and three ST1 (isolates from 2006). Both STs herein described are prevalent Typhi sequence types worldwide, and have been reported in Brazil (Tiba-Casas et al., 2018). However, our isolate 13/11 presented a MDR profile which differs from the isolates ST2 previously reported in Brazil that showed resistance only against streptomycin and sulfonamides (Tiba-Casas et al., 2018).

The isolate 14/05 was classified as ST32, a *S.* Infantis sequence type described as possibly its main allelic profile for being the most common ST of this serovar worldwide. Studies conducted in Brazil with *S.* Infatis isolated from different sources only reported the presence of ST32 in São Paulo state (Almeida et al., 2013; Monte et al., 2019). Although epidemiological studies about this serovar in Brazil lack antimicrobial resistance data, a recent study isolated *S.* Infantis from food with a different antimicrobial resistance genotype and phenotype when compared to isolate 14/05 herein studied, showing the ST32 strains being disseminated in São Paulo present different subtypes (Monte et al., 2019).

The isolate 725/16 belongs to the ST19 that has been previously reported in *Salmonella* Typhimurium isolated from food in Brazil harboring the same incompatibility groups of plasmids and the same antimicrobial resistance genes indicating this sequence type is disseminating an MDR profile among *S.* Typhimurium and monophasic variants (Monte et al., 2019).This isolate was confirmed as a Typhimurium monophasic variant due to deletions found in *fljA*, the promoter *hin,* and the regions STM2762, STM2763, STM2766, and STM2767, was closer to Spanish clones of monophasic variants. This deletion pattern has been previously described as “pattern 4” in monophasic variant samples in France isolated from food-producing animals (Bugarel et al., 2012). This genotype is more closely related to Spanish clones, although the Spanish clone lacks the *fljB* gene, and likewise the genotype described in France, the monophasic variant 725/16 harbors this gene. Previously, we have described that monophasic variants isolated in Brazil have a deletion identical to USA clones (Sales et al., 2018). Therefore, our results demonstrated that different clones of monophasic variant are circulating in Brazil. This reinforces the wide diversity presented by this serovar, and more epidemiological studies need to be taken in Brazil to understand the clones circulating within the country and their dissemination.

Although there are not abundant genomic data available regarding *Salmonella* subspecies non-*enterica,* comparing the STs herein found with the Enterobase (http://enterobase.warwick.ac.uk/species/index/senterica) we could find *Salmonella*

diarizonae ST1845 isolated from humans and wild animals in the United States, United Kingdom, and Germany, indicating this sequence type has been already correlated to human infections in other parts of the world. Although most genomic data found in Enterobase correlated with the ST1208, the sequence type found in *Salmonella salamae* 644/10, were isolated from food and environmental samples, this sequence type has also been reported in human infections in Kenya, United Kingdom, Canada, and South Africa. That indicates both sequence types of non-*enterica* subspecies herein reported present a pathogenic profile to humans.

6.2.1. Antimicrobial Resistance Genotype

Even though antibiotic susceptibility *in vitro* tests are an effective and low-cost tool, whole-genome sequencing provides all the antimicrobial resistance determinants information of a sample, which evaluates the risks of future resistance phenotypes, helps to elucidate resistance genes dissemination and improves studies about intrinsic mechanisms linked to antimicrobial resistance. Some of the antimicrobial resistance determinants found among the sequenced samples did not present their correlated resistance phenotype in the Kirby-Bauer test, indicating that these isolates present genetic features that could lead to a resistant phenotype if exposed to certain drugs.

All isolates presented efflux pump genes and efflux pump regulators (*sdtA, mdtK*, *crp, baeR, msbA, emrB, soxRS, marA,* and *hns*) besides resistance determinants against fosfomycin, elfamycin, and aminoglycosides (*eftu, bacA, glpT, uhpT,* and *aac(6')-ly*). All sequenced isolates presented streptomycin resistance phenotype, corresponding to the presence of *aac(6')-ly* gene in all isolates. Detection of multiple efflux pumps among all bacterial isolates is worrisome because their overexpression can result in multidrug resistance phenotypes (Nagakubo et al, 2002). For instance, *E. coli* mutants overexpressing the response regulator *baeR*, part of a two-component system, showed an elevated number of *mdtABCD* efflux pumps, and these cells were resistant to novobiocin and bile salt derivatives (Nagakubo et al., 2002). Another example is the *marA*, *soxRS* and *sdiA* loci, these genes are important for stress-response in bacterial cells, and when activated they can result in overexpression of efflux systems, such as *acrAB-tolC,* that have been correlated with resistance to different antibiotic classes, including beta-lactams and fluoroquinolones (Tavio et al., 2010). Therefore, an acquisition of a mutation in those loci could result in a multi-drug resistance phenotype of our samples.

While all isolates presented protein homologs of the efflux pump genes mentioned above, according to CARD the *soxRS* regulon, also found in all samples, corresponded to a variant protein correlated with the overexpression of this system. In *Salmonella* spp. and *E. coli* overexpression of the gene *soxS*, regulated by *soxR*, results in increased expression of efflux pump genes, such as *acraB*, correlated with multi-drug resistance. Although the mechanisms correlated to overexpression of these genes are still not completely elucidated, a study demonstrated that *Salmonella* spp. strains constitutively expressing *soxR* presented increased resistance against ampicillin, nalidixic acid, chloramphenicol, and tetracycline, while constitutive expression of *soxS*, caused by a *soxR* mutation, resulted in quinolone resistance (Koutsolioutsou et al., 2005).

Among the ten isolates sequenced six presented resistance only against aminoglycosides which is not linked to overexpression of *soxRS,* hence more studies would need to be conducted to confirm this overexpression and unveil the mechanisms behind it. The other four isolates presented a resistance phenotype that could be associated to the overexpression of *soxRS*, but most of them presented other genes correlated to this phenotype, thereby *soxRS* could have contributed to these isolates' resistance, but it was not the only factor. Nevertheless, the Infantis isolate 14/05 presented a resistance phenotype against amoxicillin, sulfamethoxazole/trimethoprim, and tetracycline, which did not correspond to any other genetic factors besides the presence of efflux pumps. Therefore, *soxRS* could have played a role in the tetracycline resistance phenotype of this sample, while other efflux pumps could be correlated to the non-susceptibility against sulfamethoxazole/trimethoprim.

In addition to these efflux systems shared by all isolates, three multidrug-resistance isolates (Enteritidis, monophasic variant, and Infantis) presented *golS*, an efflux pump correlated to metal resistance that is also associated to beta-lactam resistance (Pontel et al., 2007). Moreover, the monophasic variant isolate also presented *mdsAB*, another type of *tolC* dependent efflux system commonly found in *Salmonella* Typhimurium strains (Horiyama et al., 2010), besides playing an important role in the virulence of this serovar (Song et al., 2015). These efflux systems are intrinsic mechanisms presented by many gram-negative bacteria, besides being correlated with stress-response and quorum-sensing. Nevertheless, when exposed to sub-therapeutical doses of antimicrobials, this can lead to an overexpression of these systems or selection of cells carrying mutations that result in constitutive expressions of the efflux systems regulators (Tavio et al., 2010).

Apart from the efflux pump genes in the multi-drug resistant strains, other antimicrobial resistance factors were detected, and all the mechanisms correlated with those genes are summarized in Figure 12. Starting with factors correlated with fluoroquinolone/quinolone resistance, the Enteritidis 520/08, Typhi 13/11, and monophasic variant 725/16 carried one chromosomal point mutation in the *gyrA* gene, which changes the target of quinolones, thereby decreasing the bacteria's susceptibility to this class (Gouvea *et al.,* 2015). Previous studies have shown that one point mutation in this gene is usually correlated to nalidixic acid resistance (a quinolone not tested in this study), while more than one mutation in this gene is necessary to result in a fluoroquinolone resistance phenotype (Gouvea *et al.,* 2015). This corroborates with our results considering that Enteritidis 520/08 and Typhi 13/11 did not present resistance against enrofloxacin nor ciprofloxacin, while the monophasic variant presented resistance to either antimicrobials, but it carried another fluoroquinolone resistance gene called *qnrE1*.

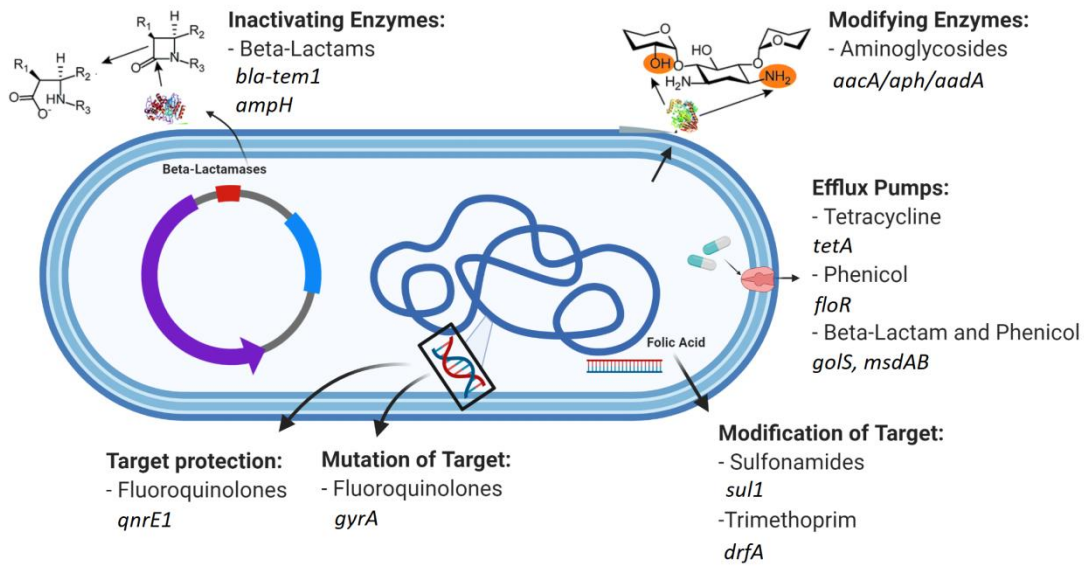


Figure 12. Mechanisms of resistance and their respective genotype found among MDR samples herein studied. Created with BioRender.com.

The *qnr* genes are plasmid-mediated quinolone resistance genes (PMQR) and play an important role in the dissemination of resistance against this antibiotic class among *Enterobacteriaceae* bacteria (Albornoz et al., 2017). This family of genes encodes a pentapeptide known for causing low-susceptibility against quinolones and fluoroquinolones by binding to the DNA gyrase preventing the antimicrobials to bind to it (Strahilevitz, 2009). The gene family's *qnrA*, *qnrB,* and *qnrS* are commonly reported in *Salmonella* spp. isolates

around the world including Brazil (Nobrega, 2016; Mendonça, 2016). However, the *qnrE1* gene herein reported belongs to a new family, previously named as *qnrB88,* firstly described in a clinical isolate of *Klebsiella pneumoniae* from Argentina (Albornoz et al., 2017). The Argentinian study showed the presence of an ISEcp1 transposase upstream the *qnrE1* gene, which is believed to have originated in the chromosome of an *Enterobacter* spp. strain. This gene has shown 100% identity with this *Klebsiella pneumoniae* (GenBank accession no. KY781949) gene and also presented the same transposon. In Brazil, *qnrE1* has been reported in *Klebsiella pneumoniae* isolated from a parrot (Cunha et al., 2017), in *Salmonella* Typhimurium isolated from industrialized products (under the previous name *qnrB88*) (Almeida et al., 2018), in *Salmonella* Enteritidis, Newport and Infantis isolated from humans (Soares et al., 2019), and in *Salmonella* Typhimurium isolated from food (Monte et al., 2019).

Analyzing the genetic context of our sample, our plasmid composition was closely related to the *S.* Typhimurium isolated from Brazilian food (Monte et al., 2019), where the *ahp* gene presented a 1035bp size in contrast to the 5'-truncated *ahp* from previous reports (Albornoz et al., 2017; Cunha et al., 2017; Soares et al., 2019). Moreover, the same studies reporting the 5'-truncated *ahp* downstream *qnrE1,* also reported this PMQR gene in IncM1 replicon type plasmids, whereas our sample only presented IncH/IncF plasmids likewise reported by Monte et al. (2019) in *S.* Typhimurium strains from food. This indicates this PMQR gene is being disseminated by more than one plasmid replicon type, and to the best of our knowledge this is the first report of a *Salmonella* spp. IncH/IncF plasmid harboring the *qnrE1* in clinical isolates from Brazil. Interestingly, all the *Salmonella* Typhimurium isolates harboring *qnrE1* reported by Almeida et al. (2018) and Monte et al. (2019) presented the same resistance genes of our monophasic isolate 725/16, which could indicate the dissemination of a multi-drug resistant plasmid in Brazil.

Another plasmid-mediated resistance gene found was the $bla_{TEM\text{-}1B,}$ which is responsible for conferring resistance to antimicrobials from the beta-lactam family. This gene was found in the monophasic variant 725/16 and Typhi 13/11, both resistant to amoxicillin, but sensitive to all other beta-lactams herein tested. The gene $bla_{TEM\text{-}1B}$ produces a TEM-1 beta-lactam enzyme capable of hydrolyzing ampicillin, amoxicillin, and other penicillins, besides early cephalosporins, but it cannot inhibit extended-spectrum cephalosporins (Blazquez et al., 1995; Babic et al., 2006), which corroborates with the phenotype of the samples. This type of beta-lactamase enzyme is responsible for most of the *Enterobacteriaceae* resistance phenotype against beta-lactams (Cantón, 2008). Although this

enzyme is not correlated with extended-spectrum cephalosporins resistance, studies have shown that mutations in TEM-1 can result in the ability to hydrolyze those antimicrobials (Blazquez et al., 1995). Hence, misuse of beta-lactams could work as a selective pressure of mutated strains contributing to the emergence of ESBL-producing strains.

Plasmid-mediated beta-lactam resistance is not the only resistance mechanism against this antimicrobial class. For instance, chromosomal encoded beta-lactamases have also been described. The Enteritidis 520/08 was the only sample presenting the ESBL phenotype among the sequenced samples, but it did not present any plasmid-mediated ESBL genes. Nevertheless, this sample presented 9 SNPs in the *ampH* gene with 5 of them being unique compared to the other samples from this study. The *ampH* gene is a beta-lactam binding protein classified as an ampC-like beta-lactamase, and it binds to penicillin G, cefoxitin, and cephalosporin C. This gene is commonly present among *Enterobacteriaceae* bacteria and its absence in *E. coli* strains have shown to alter the morphology of bacterial cells (Henderson et al., 1997). In addition to its important role in cell morphology, *ampH* has shown beta-lactamase activity at similar rates to *ampC* in previous studies (Gonzalez-Leiza et al., 2011). Mutations in class C beta-lactamases enzymes and other beta-lactam binding proteins can lead to their overexpression and result in resistance against cephalosporins without affecting bacteria's fitness (Sun et al., 2014; Berrazeg et al., 2015). Therefore, *ampH* expression levels due to mutations would need to be investigated considering not only Enteritidis 520/08 presented SNPs in this region. Moreover, other possible mechanisms and expression level of other regions should be further investigated, such as mutations in other beta-lactam binding proteins that also present beta-lactamase activity, in order to investigate the ESBL phenotype presented by Enteritidis 520/08.

In addition to the resistance genes already discussed, monophasic variant and Typhi 13/11 presented additional genes correlated to other antimicrobial classes. Besides the gene $bla_{tem\text{-}1B}$, the *Salmonella* Typhi 13/11 also presented the aminoglycoside modifying enzymes *aph(6)-Id* and *aph(3'')-lb*, besides genes encoding for enzymes in the folic acid metabolic pathway that are associated with resistance against trimetophrim *(dfrA14)* and sulfonamides (*sul2*) (Dominguez et al., 2019; Garneau-Tsodikova, 2016). Similar antimicrobial resistance profiles have been described before in Typhi isolates from Sub-Saharan Africa, differing only for the absence of *aph(3'')-lb* and for harboring another type of TEM enzyme (Park et al., 2018). The monophasic variant 725/16 isolate presented different aminoglycosides and sulfamethoxazole/trimethoprim resistance genes compared to the Typhi 13/11. The *dfrA1*

gene presented by this isolate is prevalent among isolates in Europe (El-Tayeb et al., 2017), and has been reported in *Salmonella* spp. from non-human sources in Brazil likewise the other genes *tetA, floR, sul1, aadA1,* and *aac(6')-Iaa*, presented by this sample (Monte et al., 2019; Almeida et al., 2018). These resistance genes can be found in mobile elements which explain their wide dissemination not only in Brazil but also in other continents (McMillan et al., 2019; Abatcha et al., 2018; El-Tayeb et al., 2017; Zhu et al., 2017; Adesiji et al., 2014; Stoll et al., 2012).

6.2.2. Mobile Elements

Prophages are defined as a bacteriophage genetic content inserted into the bacterial genome, and are very abundant among bacteria and play an important role in their evolution and diversity (Casjens et al., 2016). Among the samples studied, nine out of ten presented at least one P2-like prophage intact sequence, apart from the monophasic variant isolate, which presented only one lambdoid phage Gifsy-1. The P2 supercluster includes a variety of phages commonly found in *Salmonella* spp. and other *Enterobacteriaceae* bacteria, and are known for playing an important role in genetic variation of *Salmonella* spp. serovars, even though they are not uniformly distributed within them (Casjens et al., 2016). Although our study has a limited number of samples from the same serovar, our results corroborated with previous studies showing the prophage sequences are not uniformly distributed per serovar, but samples belonging to the same serovar shared at least one phage sequence. (Casjens et al., 2016)

Apart from the P2-like phages, eight samples presented lambda phages (SEN34 or Gifsy). All Typhi and *S. diarizonae* 08/16 presented SEN34, and *salamae* 644/10 and monophasic variant 725/16 presented Gifsy-1, while the Enteritidis 520/08 presented Gifsy-2. The SEN-34 phage was first described in *Salmonella* Typhi CT18, and it is commonly found among other Typhi strains (Casjens et al., 2016), although it has also been described in *S. diarizonae* (Mikalova et al., 2017), corroborating with our results.

The Gifsy phages are known for carrying important virulence genes among *Salmonella* spp. with studies showing that *Salmonella* Typhimurium lacking this phage sequence resulted in the loss of virulence in mice infection model (Figueroa-Bossi & Bossi, 2002). Although genes *gogA* and *gipA,* known for being carried by Gifsy-1 were not found in 725/16 and 644/10, the monophasic variant 725/16 carried *gogB* an anti-inflammatory effector protein that limits NFκB activation in the host's macrophages, and it is a virulence

factor harbored by Gifsy-1 phage (Pilar et al., 2012). The Enteritidis 520/08 prophage Gifsy-2 harbored an important virulence factor, the *sodC-1*, which encodes for a Cu Zn-dependent periplasmic superoxide dismutase, playing an important role in intracellular environment and contributing to bacterial pathogenicity (Uzzau et al., 2002).

Although the monophasic variant 725/16 only presented the Gifsy-1 intact phage, it also presented the partial sequence of Gifsy-2 and the gene *sodC-1* within the prophage region. As demonstrated previously in the literature, Gifsy-1 becomes essential to bacteria pathogenicity in cells carrying *sodC1* in the absence of intact Gifsy-2 (Figueroa-Bossi & Bossi, 2002). Hence, this genotype might contribute to the virulence of our isolate. The presence of important virulence factors among the prophage sequences herein described demonstrates the importance of bacteriophages not only to bacteria's diversity but also to their pathogenicity.

Along with bacteriophages, plasmids are also part of mobile elements that play an important role in bacteria evolution due to their high plasticity and modularity, where genes that play an important role in adaptation can be transferred among bacterial cells (Silva et al., 2012). Among our samples, two major incompatibility groups were found: IncF and IncH. The MDR Typhi isolate 13/11 carried only one plasmid replicon type IncFIB, which was also described in a phylogeographic study with Typhi isolates in Sub-Saharan Africa. This study has shown that isolates from Tanzania carried IncFIB plasmids harboring the same antimicrobial resistance genes as our isolate 13/11, presenting only a variation in bla_{tem} gene. While our isolate presented the TEM-1 enzyme, the African isolates presented the TEM-95/-93. Interestingly, this same genotype was also found in other samples from East Africa, but carried by IncHI plasmids, showing that dissemination of AMR genes can occur in multiple ways (Park et al., 2018). This result shows more studies are necessary to identify MDR Typhi isolates in Brazil and understand the transmission pattern correlated with IncFIB plasmids in order to mitigate its dissemination.

In addition to the Typhi isolate, the Enteritidis 520/08 also carried IncF incompatibility group plasmids, but it carried two different replicon types: the IncFII and IncFIB. In contrast to the MDR Typhi isolate 13/11 and other studies showing IncFIB plasmids harboring a variety of AMR genes in *Salmonella* spp. (Park et al., 2018; Ladely et al., 2016), the Enteritidis 520/08 did not harbor any AMR plasmid-associated genes. The IncFII+IncFIB replicon types are part of a virulence plasmid (pSEV) commonly associated

with *Salmonella* Enteritidis and also found in invasive *E. coli* strains that carry important virulence factors such as *pefABCD*, *rck,* and *spvABCDR,* all of them presented only by our Enteritidis isolate 520/08 (Garcia et al., 2019). Figure 13 shows the alignment of *Salmonella* Typhimurium LT2 virulence plasmid pSTV (95kb) in comparison with the pSEV detected in sample Enteritidis 520/08 (60kb). As previously described, both plasmids are phylogenetically close, with pSEV being derived from pSTV (Chu et al., 1999). Moreover, both plasmids harbor the virulence factors *pefABCD*, *rck,* and *spvABCDR* as shown in Figure 13. Although variations of this pSEV have been described harboring resistance genes among *Salmonella* Enteritidis and other serovars (Garcia et al., 2019; McMillan et al., 2019), Enteritidis isolates harboring pSEV without AMR genes likewise herein reported are also common (Garcia et al., 2018; McMillan et al., 2019). This result shows the importance of plasmids not only to the dissemination of AMR genes but also important virulence factors.

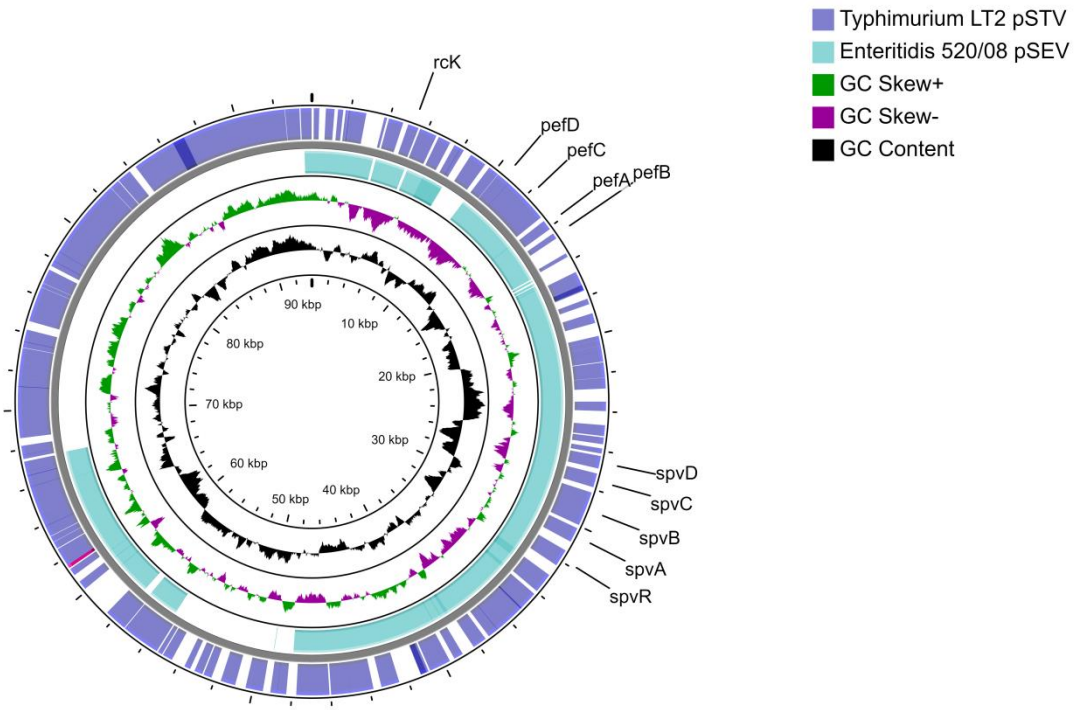


Figure 13. Alignment of the *S.* Typhimurium LT2 virulence plasmid pSTV (in purple) with the virulence plasmid pSEV present in sample Enteritidis 520/08. The mainly virulence factors presented by the virulence plasmids are highlighted, and the GC skew and content are shown in the inner rings. Created with CGView.

The MDR monophasic variant 725/16 was the only one to carry plasmids from both incompatibility groups IncF (IncFIA) and IncH (IncHI2 and IncHI2A). Both replicon types have been reported in *Salmonella* spp. isolates presenting AMR genes, including genes found

in our isolate (McMillan et al., 2019; Wong et al., 2016; Chen et al., 2018). Although both replicon types might play an important role in AMR genes dissemination, HI2 plasmid type is prevalent among MDR *Salmonella* spp. samples (Chen et al., 2018). Moreover, studies have reported the co-occurrence of *qnr* and bla_{tem} genes in the same plasmid replicon type HI2 type among *Salmonella* spp. isolates (Gonzalez & Araque, 2013; Perez-Moreno et al., 2013; Jiang et al., 2014). In Brazil samples of *Salmonella* Typhimurium isolated from food presented the same genotype of our sample 725/16 and also carried IncFIA, IncHI2, and IncHI2A replicon types (Monte et al., 2019). Nevertheless, this last study did not present the whole genome sequence of the plasmid; thereby it is not possible to compare our draft genome and determine rather the AMR genes are harbored by the IncF or IncH replicon type. Hence, it would be necessary to fill the genome-sequence gaps and determine the plasmid content from our sample in order to elucidate the dissemination of antimicrobial resistance genes through this vector.

6.2.3. Virulence Factors

The presence of SPIs was similar among isolates from the same serovar corresponding to previous results showing conservation of SPIs presence among strains from the same serovar (Amavisit et al., 2003). Moreover, SPI-1 through SPI-5 were conserved among all *enterica* subspecies samples, with minor deletions mostly of hypothetical proteins, or regions that are not correlated with T3SS. The conservation of these islands reinforces the importance of these regions for the pathogenicity of *Salmonella* subspecies *enterica*. Although SPI-2/4/5 did not present any deletions among *enterica* samples, the typhoid isolates did not present the gene *avrA* located in SPI-1 and *sugR* located in SPI-3. The absence of *avrA* among typhoid isolates has been previously reported in numerous strains, and there are discussions about the lack of *avrA* being linked to macrophage evasion and systemic infections because this gene is an immunomodulatory protein that regulates apoptosis (Giacomodonato et al., 2014). The absence of *sugR* in SPI-3 has been previously reported in many different serovars of *Salmonella enterica*. Although its functions is still not elucidated, this region was defined as the most variable region among *S. enterica* serotypes and it does not seem to jeopardize the virulence of bacteria (Amavisit et al., 2003).

Among genes from the SPI-3 the *mgtCB* cluster was found in all isolates, and it is responsible for encoding an Mg^{2+} uptake system essential for intracellular surveillance (Hensel, 2004). Additionally, the SPI-5 was conserved in all *S.* subspecies *enterica* isolates.

This island plays an important role in pathogenicity because it encodes effector proteins from the T3SS of SPI-1 and SPI-2. Genes encoded by this region include PipA, PipB, and SopB. PipA plays a role in the development of systemic infections, while the last two are SPI-2 effector proteins (Hensel, 2004).

Moreover, the SPI-6 was only present among *enterica* subspecies isolates, corroborating with previous phylogenetic studies showing that SPI-6 was acquired by subspecies *enterica* during the evolution process of this genus (Desai et al., 2013). However, this region presented divergences among the *enterica* isolates, and one important gene homologous to the *vgrG* in *Pseudomonas* spp. was partially deleted in the Enteritidis 520/08. This gene plays an important role in competition with the host's microbiota likewise the proteins from *rhs* family, also deleted in the Enteritidis, Typhi, and Infantis isolates. Hence, a deletion within this region in SPI-6 could jeopardize the isolates invasion process (Navarro-Garcia et al., 2019). However, there were SPI-6 regions conserved among all *enterica* isolates such as the fimbriae genes *saf*, its presence was correlated with invasive *Salmonella* spp., but its deletion did not affect the virulence phenotype of mutant strains most likely because *Salmonella* spp. possess several fimbrial genes that migh have a redundant function (Antony et al., 2018; Łaniewski et al., 2017). Therefore, despite minor deletions found within SPI-1/3/6, there are conserved regions correlated with human-infections among all the *Salmonella* subspecies *enterica* isolates.

Another important frimbriae cluster found among our isolates was the *lpf*, which was present only in non-typhoidal subspecies *enterica* samples, and it produces longer polar frimbriae. This fimbriae cluster has been correlated with virulence in humans (Lamas et al., 2018), and *lpf* mutant strains are not capable of forming biofilm in tissues showing the importance of this cluster in adhering to the cell surface (Ledeboer et al., 2006).

The presence of SPI-8 was shared by Enteritidis and Typhi. This island role is not completely understood, but it has been described as being specific to *S.* Typhi. However, an integrase is encoded within this region, indicating possible mobility (Hensel, 2004). Our findings corroborate with this information, considering all Typhi isolates presented an intact SPI-8, but this region was also detected in Enteritidis 520/08, reinforcing the possible mobility of this element and a wider distribution among serovars.

Besides the regions shared by subspecies *enterica* samples, there were also virulence factors unique to each isolate. As expected, Enteritidis 520/08 was the only one to present the

fimbrial gene *sefA,* which encodes fimbriae SEF (*Salmonella* Enteritidis Fimbriae) that are specific to serovars from group D1. These fimbriae are involved in bacterial adhesion to host's Peyer's plate during the infection process (Mendonça, 2016).

Additionally, isolate 520/08 carried genes *rck, spvRABCD,* and *pefABCD* that are found within the *Salmonella* Virulence Plasmids (pSV), which are part of the incompatibility group IncF, also detected in our sample. The *spv* region is essential for bacterial survival inhibiting neutrophils and macrophages, and it has been shown that Typhimurium strains with *spv* operon were more virulent compared to those lacking this region (Silva et al., 2017). The gene *rck*, also located in pSV, helps to modulate the host's immune response, while the *pef* fimbrial operon induces the host's inflammatory response and helps with epithelial cell's adhesion (Silva et al., 2017). Another important virulence factor detected in Enteritidis 520/08 was the prophage-encoded *sodC1*, also detected in the monophasic variant 725/16, and as previously mentioned, this gene protects the bacteria against reactive oxygen species playing an important role in host's colonization.

The monophasic variant 725/16 presented another prophage-encoded virulence factor, the *gogB* gene which is a T3SS that contributes to the modulation of the host's immune response (Pilar et al., 2012). In addition to *gogB*, the 725/16 isolate carried *gtrA*, a glucosyltransferase that modifies the O-antigen, promoting an immune evasion mechanism and defense against bacteriophages (Davies, et al., 2013).

Another virulence factor carried by this isolate was an invasin A from *Yersinia* spp. that was also present in the Typhi 13/11. This gene is a type III secretion system of *Yersinia* spp., and studies with mutants of *Yersinia* spp. lacking invasins and *E. coli* expressing those same invasins have shown that they play an important role in the inflammation and infection process (Gillenius & Urban, 2015). The T3SS in *Yersinia spp.,* as well as the T3SS in *Salmonella* spp., encodes important genes correlated with invasion and survival of bacterial cells and part of these proteins form a needle structure that translocates effector proteins (Gillenius & Urban, 2015). Some of these virulence factors from Yersinia spp. are homologous to *Salmonella* spp. (Marcus et al., 2000), and they are encoded by virulence plasmids, thereby our samples could have acquired this gene horizontally.

Also, the monophasic variant isolate 725/16 harbored *ibeB*, found as well in *S. salamae* 644/11 isolate. This gene is important to the invasion of brain endothelial cells, and it has been described in pathogenic *E. coli,* such as the neonatal meningitis *Escherichia coli*

(NMEC) (Wang et al., 2012). Moreover, this gene is also found among avian pathogenic *E. coli* (APEC), and APEC strains lacking this gene have a poor ability to colonize and invade their host cells (Wang et al., 2012). Therefore, this gene might have been horizontally acquired by isolate 725/16 and *S. salamae* from APEC other pathogenic *E. coli.*

The wide diversity of virulence factors and SPIs found among our samples shows that invasive *Salmonella* spp. has different mechanisms and genes involved during the colonization process. Moreover, our isolates harbored virulence factors from *Salmonella* spp. and other species linked to mobile elements, showing horizontally acquired genes are not only important to antimicrobial resistance but also pathogenicity, increasing the diversity of pathogenicity factors even among strains from the same outbreak likewise our 2006 Typhi isolates.

Salmonella spp. is part of the natural gut microbiota in reptiles, including subspecies other than *enterica,* such as *diarizonae* and *salamae*. These two last subspecies are not commonly isolated from human infections, but since the increasing number of reptiles as pets, outbreaks caused by unusual *Salmonella* subspecies, especially *arizonae, diarizonae,* and *salamae,* have been reported in different continents (Giner-Lamia et al., 2019, Gerlach et al., 2017; Lamas et al., 2018). Among our isolates, there were two *S. diarizonae,* one isolated from blood (08/16) and the other one from urine (30/10), besides one *S. salamae* isolated from feces. To the best of our knowledge, there are no reports of human infections caused by non-*enterica* subspecies in Brazil. Therefore, we further investigated the virulence genotype and phenotype of non-*enterica* isolates.

Regarding the *Salmonella* Pathogenicity Islands (SPIs), all three samples presented the intact C63PI region and the SPI-2, besides presenting most genes, especially those encoding T3SS proteins, of SPI-1 and SPI-3 well conserved. The conservation of SPI-1/2/3 has been shown in previous studies analyzing genomes from different *Salmonella* subspecies, hence reinforcing the importace these islands possess to pathogenicity of this genus (Desai et al., 2013). Moreover the two *diarizonae* isolates also presented the SPI-13. The function of SPI-13 is still not completely elucidated, but it is known that SPI-13 plays an important role in metabolizing substances found in the gastrointestinal tract, and strains lacking this region presented lower virulence in the murine model (Elder et al., 2018). While the centisome C63PI harbors genes *sitABCD* that encodes proteins related to iron transport (Ashari et al., 2019).

In addition to the SPIs detected, the isolate *S. salamae* 644/10 presented unique virulence factors when compared to the other isolates. For instance, 644/10 presented the enterocyte effacement (LEE) locus homologous to *E. coli* OH157:H7, whose presence is exclusive to subspecies *salamae* among the genus *Salmonella* (Desai et al., 2013). This sample presented 18 out of 24 known genes from the LEE locus which is associated with intestinal epithelial cells adhesion leading to diarrhea (Jarocki et al., 2020). Furthermore, it presented ACE-like genes from *E. coli* (Type VI secretion system proteins), *ibeB,* and *usp*. These genes are presented by LPEC, NMEC, APEC and UPEC *E. coli* strains (Tian et al., 2017; Wang et al., 2012; Nipic et al., 2013). The colicin *usp* is a bacteriocin associated with DNA damage of the urinary tract cells, while *ibeB* play a role in humans epithelial brain cells invasion, showing the isolate 644/10 might be able to invade other cells rather than epithelial cells from the intestinal tract (Wang et al., 2012; Nipic et al., 2013).

Despite the presence of the invasion genes discussed above, the isolate 644/10 was the only one lacking some important virulence factors from SPI-5 likewise *pipAB*, an important effector protein correlated with the development of systemic infections, and *sopB,* a T3SS important for invasion. The absence of SPI-5 genes has been described in *S.* Sofia, a non-virulent serovar of *S. salamae* commonly isolated in Australian livestock (Gan et al., 2011). Moreover, studies have shown the loss of SPI-5 was part of the evolution process of this subspecies, and it could be correlated with its attenuated phenotype (Desai et al., 2013). The *pipAB* region was partially deleted in all the *diarizonae* isolates, which could result in the production of a truncated protein, and consequently a loss in their invasion efficiency.

Furthermore, there were virulence factors presented by all subspecies non-*enterica* isolates, such as *cdtB* part of the *cdtABC* gene cluster, which has been previously shown to be correlated with the production of a CDT toxin only found in *arizonae* and *diarizonae* subspecies (Desai et al., 2013). However, not only our *diarizonae* and *salamae* isolates harbored the *cdtB* gene, but also all *Salmonella* Typhi isolates, differing from previous results that show this gene only among *diarizonae* and *arizonae* samples (Desai et al.,. 2013).

The virulence factors found among both *S. diarizonae* isolates were similar, with the exception of the gene *escS* from the LEE *E. coli* locus presented only by isolate 08/16, and the *pilQRS* from *Yersinia* spp. only found in isolate 30/10. This similarity could be correlated with the conservation of virulence factors among strains from the same subspecies and serovars likewise both *diarizonae* isolates. Additionally, these two isolates carried several

virulence factors associated with other bacteria genus. The first one was proteins from the T6SS of *E. coli* and *Klebsiella* spp. associated with bacteria competition (Navarro-Garcia et al., 2019; Barbosa & Lery, 2019). The non-*enterica* isolates did not harbor the SPI-6, an important island for bacterial competition that encodes T6SS proteins, and gained by *Salmonella* subspecies *enterica* during the evolution process of this genus (Lamas et al., 2018). Therefore, the presence of T6SS of *E. coli* and *Klebsiella* spp. in both *diarizonae* isolates might have compensated the absence of SPI-6, allowing these bacteria to colonize their hosts successfully. The second one was the virulence factor *pilW* from *Yersinia* spp., part of a gene cluster encoding type IV pili and it is homologous to a *Yersinia pestis* putative transposase, indicating a possible horizontal transfer (Collyn et al., 2002). *Yersinia* spp. lacking type IV pili are attenuated in mice, and play an important role in adhesion and of host cells. Studies have shown that *pilW* is part of *pil* operon highly homologous to the type IV pili in *Salmonella* spp., but *pilW* is the only gene among this operon not present in *Salmonella* strains. Moreover, studies with *E. coli* mutant strains have shown that bacteria lacking *pilW* are still able to synthesize the pili; thereby its function still needs to be elucidated (Collyn et al., 2002).

Besides the *pilW,* other *Yersinia* spp. genes correlated with iron acquisition and uptake (yersiniabactin) were present in both isolates. These genes are located on a mobile element in *Yersinia* spp. called the High-pathogenicity Island (HPI) that can be horizontally transferred among bacteria and have been reported among the *Enterobacteriaceae* family (Rakin et al., 2012).

6.3. Virulence Phenotype of non-*enterica* Samples

Considering all the virulence factors found among non-*enterica* isolates, *in-vivo* and *in-vitro* assays were performed in order to better characterize their virulence phenotype. The *Galleria mellonella in-vivo* assay showed an invasive phenotype demonstrated by the non-*enterica* subspecies samples. When compared to the virulent control UK-1, the non-*enterica* samples killed all larvae within 24h at the concentration of 10^3 CFU/µl, while UK-1 did not kill any larvae within the same period at the same concentration. Using the dosage 10^2 CFU/µl the isolates UK-1 and *S. diarizonae* 30/10 did not kill any larvae, while isolates 644/10 and 08/16 killed 6, and 8 insects respectively. Using the Mantel-Haenszel approach to compare the samples survival rates there was no significant difference between isolates 08/16 and 644/10 survival curves ($p = 0.3$) injecting 10^2 CFU/µl. Nevertheless, when comparing

these two isolates with the virulent control UK-1 and the *S. diarizonae* 30/10 there was a significant difference ($p < 0.05$) of their survival curves using the bacterial concentration of 10^2 CFU/µl. This result shows that all non-*enterica* samples tested were more virulent than UK-1 using this infection model. Furthermore, the isolates 08/16 (*S. diarizonae*) and 644/10 (*S. salamae*) were more virulent than the isolate 30/10 (*S. diarizonae*) according to this assay.

The low survival rates of *Galleria mellonella* infected with the subspecies non-*enterica* compared to the UK-1 strain could be correlated with the virulence factors presented by them, such as the *cdtB* gene encoding a CDT toxin, which is not present in UK-1. *Salmonella* spp. strains carrying this gene present the lowest survival rates in the *Galleria mellonella* model, as demonstrated by other studies (Card et al., 2016). Comparing the results of the *Galleria mellonella* killing assay herein presented with other *Salmonella* spp. reference strains and isolates studied in the literature, our samples presented a higher mortality rate in a shorter time-period and lower concentration than an *S.* Typhimurium ATCC 14028, an invasive *S.* Infantis isolated from owls in Brazil (Fuentes-Castillo et al., 2019), an S. Typhimurium NCTC 12023 (Bender et al., 2013) and the Typhimurium strain SL1344 (Viegas et al., 2013).

The significant difference of larvae survival rates among *diarizonae* isolates 30/10 and 08/16 needs further investigation to better characterize possible other deletions or insertions presented by both among known SPIs. Even though VFDB results and SPIs diversity were not significantly different between them, other virulence factors herein not analyzed could be correlated with this difference in pathogenicity. Furthermore, even though there were deletions in SPI-1 among all non-*enterica*, their absence might have been compensated by the presence of other virulence factors considering many genes within this region present a redundant function (Haraga et al., 2008).

In addition to the killing assay in *Galleria mellonella*, the J774 macrophage invasion and survival assay were performed. In comparison with the reference strain Typhimurium ATCC 14028, the non-*enterica* samples did not present significant differences, thereby they were able to invade and survive within the macrophage cells. Our results differed from previous studies showing that *S. diarizonae* strains were able to adhere to J774 cells, but their invasion efficiency was poor compared to ATCC 14028 (Katribe et al., 2009). Additionally, a study conducted in Australia showed that *S. salamae* serovar Sofia presented lower invasion

levels than a virulent Typhimurium strain and it was not able to survive inside J774 macrophages, which also diverges from results herein reported (Gan et al., 2011).

Despite the absence of SPI-6 and SPI-5 (only in *salamae*), and deletions among SPI-1/3/4, which are important for invasion, surveillance, and competition, all subspecies non-*enterica* samples from our study were able to cause infections in humans, invade and survive within mice macrophages, besides leading to higher mortality rates than pathogenic strains in *Galleria mellonella* model. The *diarizonae* 08/16 was isolated from a human blood sample, and caused the highest mortality rates during the *in-vivo* assay herein performed. However, it did not present many differences regarding virulence factors analyzed when compared to the *diarizonae* 30/10, which was isolated from a human urine sample and presented the lowest mortality rates in the killing assay. The *salamae* 644/10 lacked important virulence factors that all other samples carried, such as *sopB* and *pipAB*, but presented several other virulence factors correlated to invasion. Moreover, it was more pathogenic than UK-1, and the isolate 30/10 according to the *Galleria mellonella* assay results.

The subspecies *salamae* and *diarizonae* are linked to reptile-associated Salmonellosis. Although they can infect humans, they usually affect infants or immunocompromised individuals (Giner-Lamia et al., 2019; Gerlach et al., 2017). Information about the patient's health conditions and age could not be provided to us, hence limiting our study. However, further investigations to better understand the virulence factors and invasion mechanisms of those subspecies are important to avoid outbreaks and the emergence of invasive *Salmonella* spp. strains. Therefore, we intend to perform more *in-silico* investigations to better characterize the deletions and insertions within SPIs and other virulence factors compared to other subspecies non-*enterica* strains and invasive *Salmonella* subsp. *enterica* presented in the NCBI database. Moreover, the phylogenetic analysis of these samples will be conducted to understand the evolutionary relationship of our samples with other samples from the same subspecies, and *enterica* subspecies isolated from humans and animals also presented in the NCBI database.

7. Conclusions and Future Perspectives

In conclusion, a higher proportion of the *Salmonella* spp. isolates herein studied have shown resistance against beta-lactams and aminoglycosides. Among the 810 isolates evaluated only 71 presented a multi-drug resistance profile, and although this represents a low percentage of MDR isolates, their presence in the state of São Paulo is a cause of concern to our public health system. Moreover, the presence of ESBL-producers, fluoroquinolone-resistant and colistin-resistant isolates represents a threat to human public health. Even though those profiles were present at low rates, the genes responsible for the correspondent phenotype can be present in mobile elements which could lead to dissemination of this antimicrobial resistance among *Salmonella* spp and other bacteria in Brazil.

Analyzing the genomic data of the selected samples we could conclude there are antimicrobial resistance determinants that could be intrinsic to *Salmonella* spp. considering they were found even among susceptible strains from different serovars and subspecies. Most of those factors were efflux pumps that have the ability to export several antimicrobial classes resulting in a resistant phenotype when overexpressed. This result reinforces the importance of antimicrobial's controlling measures and genomics studies to mitigate selection of resistant strains and comprehend the prevalence and mechanisms of resistance in bacterial strains circulating in Brazil. Among the MDR strains, including one Typhi isolate, we could detect resistance genes close to mobile elements and the presence of different plasmid replicon types, which indicates their resistance genes could be disseminated among other samples. Moreover, the MDR samples also carried virulence factors linked to mobile elements showing not only resistance factors can be disseminated.

The three Typhi samples isolated in a 2006 outbreak presented divergences among prophages and fimbriae genes, showing that even strains from the same sequence type isolated during an outbreak can present divergences and prophages play an important role in it. We intend to further investigate the phylogenetic relationships with the samples from this outbreak with other Typhi isolated in Brazil and other countries in order to understand the dissemination and evolutionary tie of this pathogen around the globe.

The non-*enterica* isolates presented important virulence factors correlated to invasion and intracellular surveillance, and they also presented several virulence factors of other invasive *Enterobacteriaceae*. Despite the absence of intact SPIs that are essential to *Salmonella* spp. pathogenicity, our samples still showed a virulent phenotype in *in-vivo* and

in-vitro tests when compared to invasive *Salmonella* Typhimurium reference strains. This data shows non-*enterica* subspecies have the potential to be invasive and cause disease, thereby more studies on their pathogenicity should be performed besides better monitoring of enteric diseases along the country. Further *in-silico* studies will be conducted with the non-*enterica* samples in order to understand in detail deletions and insertions among SPIs when compared to online databases. We intend to perform phylogenetic analysis to trace correlations between our samples and other *Salmonella* spp. from different subspecies and isolation sources.

8. References

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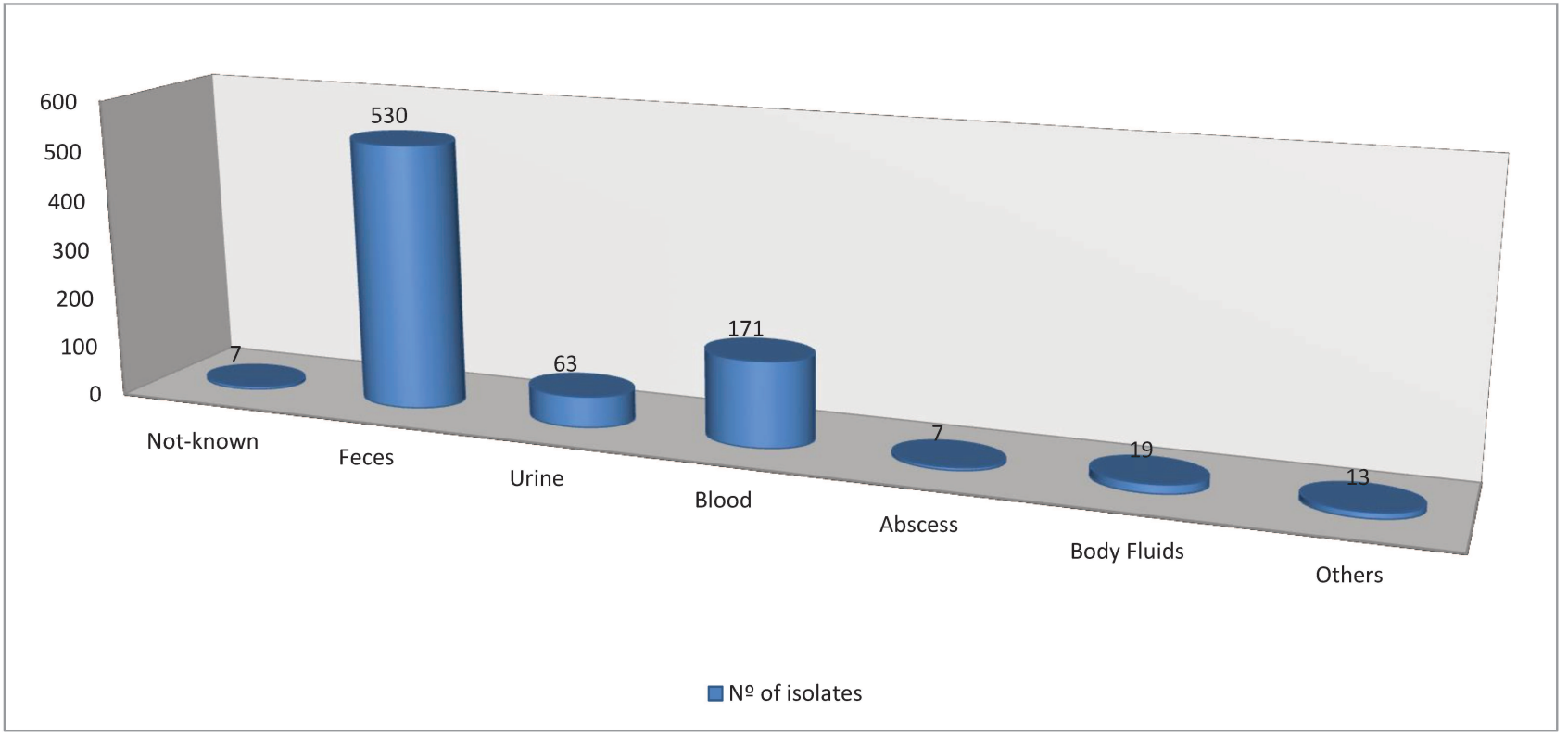
Appendix 01. Serovars and the respective antigenic formulae of each isolate herein studied.

Antigenic formulae	**Serovar Name**	**Nº of Isolates**
Not-determined	*Salmonella* spp.	70
1,4,5,12:r:-	-	1
4,12:b:-	-	1
4,5,12:eh:-	-	1
1,4,[5],12:i:-	Monophasic Variant	66
4,5,12:i:e,h:-	-	1
42:r:-	-	2
6,7,:y:-	-	1
6,7:-:1,5	-	3
6,7:r:-	-	1
6,7:Z10:-	-	1
6,8:e,h:-	-	1
6,8:i,h:-	-	1
6,P:e,h:-:	-	1
61:C:-	-	1
61:i:z	-	1
8,20:z4,z23:-	-	1
9,12	-	1
9,12:-:-	-	1
9,12:-:1,5	-	1
1,4,[5],12:f,g,s:[1,2]	Agona	5
8,20:Z_4Z_{24}:-	Albany	1
3,{10}{15}{15,34}:e,h:1,6	Anatum	1
6,7,12:y:1,5	Bareilly	3
6,7,14:e,h:e,n,z_{15}	Braenderup	3
4,[5],12:l,v:e,n,z_{15}	Brandenburg	2

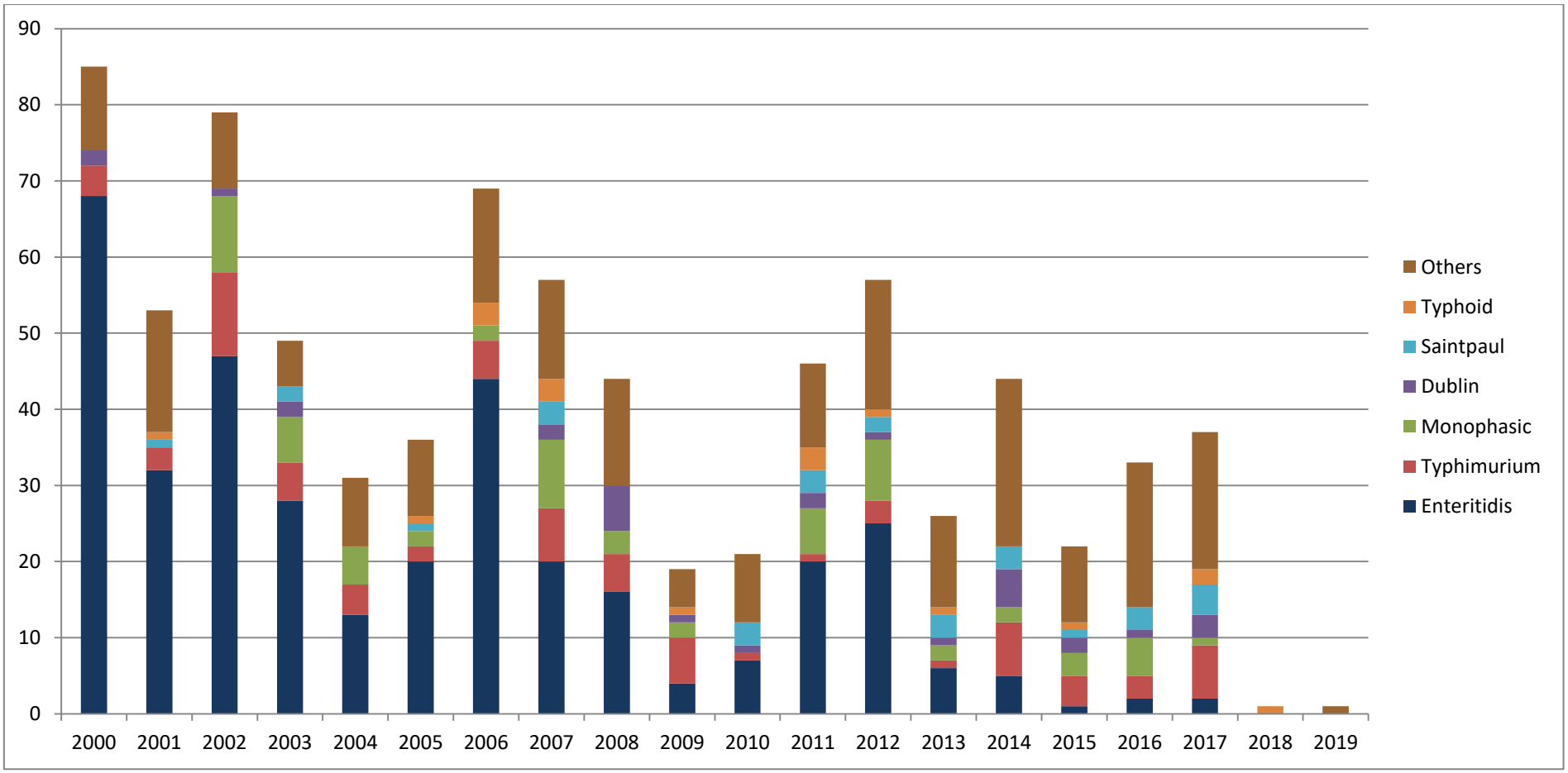
1,4,12,27:z_{29}:-	Bredeney	2
8,20,:z_4,z_{23}	Corvallis	5
61:z:c35	subspecies *diarizonae*	2
1,9,12[Vi]:g,p:-	Dublin	30
1,9,12:g,m:-	Enteritidis	360
3,{10}{15}{15,34}:l,v:1,7	Give	4
6,8:z_{10}:e,n,x	Hadar	1
1,4,[5],12:r:1,2	Heidelberg	1
6,7,14:r:1,5	Infantis	10
1,9,12:l,z_{28}:1,5	Javiana	10
8,20:i:z_6	Kentucky	1
6,7,14:d:l,w	Livingstone	1
3,{10},{15}:l,v:1,6	London	1
6,8:d:1,5	Manhattan	1
6,7,14:z_{10}:e,n,z_{15}	Mbandaka	1
1,9,12:a:1,5	Miami	2
6,7,14,[54]:g,m,[p],s	Montevideo	1
6,8:d:1,2	Muenchen	11
6,8,20:e,h:1,2	Newport	22
6,7,14:b:l,w	Ohio	2
6,7,14:m,t:[z_{57}]	Oranienburg	11
6,7,14:a:e,n,x	Oslo	1
1,9,12:l,v:1,5	Panama	18
1,4,[5],12:b:1,2	Paratyphi B	3
1,13,22:z:1,6	Poona	1
6,7,14:f,g:-	Rissen	1
11:r:e,n,x	Rubislaw	1
1,4,[5],12:e,h:1,2	Saintpaul	29

42:r:-	subspecies *salamae*	1
1,4,[5],12:e,h:e,n,z_{15}	Sandiego	2
1,4,12,27:d:1,7	Schwarzengrund	4
9,12[Vi]:d:-	Typhi	**15**
1,4,[5],12:i:1,2	Typhimurium	79
1,13,23:z:l,w	Worthington	2
16:c:l,w	Yoruba	2
Total Samples		810

Appendix 02. Number of isolates from each source of isolation.



Appendix 03. Total isolates per year divided between the five most prevalent serotypes, typhoid isolates, and other serotypes.



Appendix 04. Results of the alignment of the gene *ampH* using the reference strain *Salmonella* Typhimurium LT2 as query compared to all isolates herein studied according to BLASTn.

Query: Salmonella enterica subsp. enterica serovar Typhimurium str. LT2, complete genome. Query ID: NC_003197.2 Length: 1201

>Sbj01: monophasic variant 725/16

Score:2218 bits(1201), Expect:0.0,

Identities:1201/1201(100%), Gaps:0/1201(0%), Strand: Plus/Minus

>Sbj02: Enteritidis 520/08

Score:2169 bits(1174), Expect:0.0,

Identities:1192/1201(99%), Gaps:0/1201(0%), Strand: Plus/Minus

>Sbj03: Infantis 14/08

Score:2135 bits(1156), Expect:0.0,

Identities:1186/1201(99%), Gaps:0/1201(0%), Strand: Plus/Minus

>Sbj04: Typhi 13/11

Score:2130 bits(1153), Expect:0.0,

Identities:1185/1201(99%), Gaps:0/1201(0%), Strand: Plus/Plus

>Sbj05: Typhi 303/06; Typhi 328/06; Typhi 385/06

Score:2130 bits(1153), Expect:0.0,

Identities:1185/1201(99%), Gaps:0/1201(0%), Strand: Plus/Plus

>Sbj06: *diarizonae* 08/16; *diarizonae* 30/10

Score:1941 bits(1051), Expect:0.0,

Identities:1151/1201(96%), Gaps:0/1201(0%), Strand: Plus/Plus

>Sbj07: *salamae* 644/10

Score:2030 bits(1099), Expect:0.0,

Identities:1167/1201(97%), Gaps:0/1201(0%), Strand: Plus/Minus

```
Query  428900  TAACCAAATCGTTAATCCCGTCGCTCATATTGGTAAAACGAGTGAGGGGGGAACGGGTTA  428959
Sbj01  20859   ............................................................  20800
Sbj02  312739  ....................................C.....A.............  312680
Sbj03  38628   ....................................C...................  38569
Sbj04  66694   ....................................G...................  66753
Sbj05  127687  ....................................G...................  127746
Sbj06  136020  .............A.................T.............T........  136079
Sbj07  220427  .............A..............................A..........  220368

Query  428960  CCACCACAAAAGCGCCAATATTATATTGCGGGATCATCGCCATATAGGTAATAAAACCGC  429019
Sbj01  20799   ............................................................  20740
Sbj02  312679  .G..........................................................  312620
Sbj03  38568   .G........................T...........................  38509
Sbj04  66754   .G........................T...........................  66813
Sbj05  127747  .G........................T...........................  127806
Sbj06  136080  .G........................T...........................  136139
Sbj07  220367  .G........................T...........................  220308
```

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Query  429020  CGCCGCCTCCTGTTTTCTGGATAATGCCAGGACGTCCGTCTTTTGGCGCCATATAGACCC  429079
Sbj01  20739   ............................................................  20680
Sbj02  312619  ....T.......................................................  312560
Sbj03  38508   ............................................................  38449
Sbj04  66814   ..........C.................................................  66873
Sbj05  127807  ..........C.................................................  127866
Sbj06  136140  .......G..C.................................................  136199
Sbj07  220307  ..........C.................................................  220248

Query  429080  AACCCAGACCGAGCGCGTCGGCTTTACCCGGTACGTCCATCCCGATGATTTTCTTTAACT  429139
Sbj01  20679   ............................................................  20620
Sbj02  312559  ............................................................  312500
Sbj03  38448   .G.........................................A..............  38389
Sbj04  66874   .G.........................................................  66933
Sbj05  127867  .G.........................................................  127926
Sbj06  136200  .G...........T............................A..C.....T....G..  136259
Sbj07  220247  .G.........................................T..........G..  220188


Query  429140  GTATGCGCTGATAAATCAACGTTTGCATACGATCGGCCTGTTGGCTACGGTGATAGAAAT  429199
Sbj01  20619   ............................................................  20560
Sbj02  312499  ..G.........................................................  312440
Sbj03  38388   ..G.........................................................  38329
Sbj04  66934   ..GC.....................................A...................  66993
Sbj05  127927  ..GC.....................................A...................  127986
Sbj06  136260  .CG.....................................A...................  136319
Sbj07  220187  ..G.....................................A...................  220128

Query  429200  CTGACGACAGATACTGTTGCATCCAGCGCATCATATCTCCTGGCGTAGAATAGACCCCAC  429259
Sbj01  20559   ............................................................  20500
Sbj02  312439  ............................................................  312380
Sbj03  38328   ............................................................  38269
Sbj04  66994   ........................................................T..G.  67053
Sbj05  127987  ........................................................T..G.  128046
Sbj06  136320  ....................................................A.....G.  136379
Sbj07  220127  ....T.................................................G.  220068
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Query  429260  CGCTGCCAATGGCTGCCAGCGTATTATTACAGGGGCTTGCGCCTTTTTCTGCCACCATCA  429319
Sbj01  20499   ............................................................  20440
Sbj02  312379  ............A...........................................T....  312320
Sbj03  38268   ............A...........................................  38209
Sbj04  67054   ............A..............................C............  67113
Sbj05  128047  ............A..............................C............  128106
Sbj06  136380  .......G..A..............................C............  136439
Sbj07  220067  ....A....................................C............  220008

Query  429320  GACGCTTACACTGATCGGGAGAAGGGGTAAACGTGGTGTCTTTCATTCCCAGCGGGCGGG  429379
Sbj01  20439   ............................................................  20380
Sbj02  312319  ............................................................  312260
Sbj03  38208   ............................................................A.  38149
Sbj04  67114   ....................G.......................................  67173
Sbj05  128107  ....................G.......................................  128166
Sbj06  136440  ....................G..A....................................  136499
Sbj07  220007  .G..................G.......................................  219948


Query  429380  TAATCTTTTCTTCAAACAGTTGCGTATAAGGTTTGCCGGAGGCGGTCGCCAGCGCGTCGG  429439
Sbj01  20379   ............................................................  20320
Sbj02  312259  ............................................................  312200
Sbj03  38148   ............................................................  38089
Sbj04  67174   ............................................................  67233
Sbj05  128167  ............................................................  128226
Sbj06  136500  ............................................................  136559
Sbj07  219947  ............................................................  219888

Query  429440  CTAACAGGTCGAACGCCAGATTGGAATAGGCCGCCTGTGAACCGGGCGCGACTTTTAACG  429499
Sbj01  20319   ............................................................  20260
Sbj02  312199  ............................................................  312140
Sbj03  38088   ..................................C.........................  38029
Sbj04  67234   ............................................................  67293
Sbj05  128227  ............................................................  128286
Sbj06  136560  .......A..............................C..G..C..T..T........G.  136619
Sbj07  219887  ..................................A..G..T.....T........G.  219828
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Query  429500  TTGCTGTGGAGAGCCAGTTCCAGCGTTGTTCGCGGGTTGGCCAGACAAATACCGGACGAT  429559
Sbj01  20259   ............................................................  20200
Sbj02  312139  ............................................................  312080
Sbj03  38028   ............................................................  37969
Sbj04  67294   ............................................................  67353
Sbj05  128287  ............................................................  128346
Sbj06  136620  CC.........T.................................................  136679
Sbj07  219827  ....C.......T.................................................  219768

Query  429560  GCGCCGCGCCGCCGGGCTGTTCTCGCGGAAGCGCGCTGGTGTGGGTCGCTAAGTTGACCA  429619
Sbj01  20199   ............................................................  20140
Sbj02  312079  ............................................................  312020
Sbj03  37968   ............T...............................T.............  37909
Sbj04  67354   ............................................................  67413
Sbj05  128347  ............................................................  128406
Sbj06  136680  ........................................A...................  136739
Sbj07  219767  ............A...........................A...................  219708


Query  429620  ACGTAATCGGCGTGCCTTGATACGTCGGGACACGCGCGCCGGGAGGCGCATATTTGCTGA  429679
Sbj01  20139   ............................................................  20080
Sbj02  312019  ..............................T.............................  311960
Sbj03  37908   ..............................T.............................  37849
Sbj04  67414   ..............................T.............................  67473
Sbj05  128407  ..............................T.............................  128466
Sbj06  136740  G.............................T............G...........A.  136799
Sbj07  219707  GA............................T............G..............  219648

Query  429680  GAGGATCGTTTAATTTTACCGTTCCCTGATCGAGCAATTTTACCAACATTTCACTGGTCA  429739
Sbj01  20079   ............................................................  20020
Sbj02  311959  ............................................................  311900
Sbj03  37848   ............................................................  37789
Sbj04  67474   .C..........................................................  67533
Sbj05  128467  .C..........................................................  128526
Sbj06  136800  ........................................C...................  136859
Sbj07  219647  ............................................................  219588
```

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Query  429620  ACGTAATCGGCGTGCCTTGATACGTCGGGACACGCGCGCCGGGAGGCGCATATTTGCTGA  429679
Sbj01  20139   ............................................................  20080
Sbj02  312019  ..............................T.............................  311960
Sbj03  37908   ..............................T.............................  37849
Sbj04  67414   ..............................T.............................  67473
Sbj05  128407  ..............................T.............................  128466
Sbj06  136740  G.............................T............G...........A.  136799
Sbj07  219707  GA............................T............G............  219648

Query  429680  GAGGATCGTTTAATTTTACCGTTCCCTGATCGAGCAATTTTACCAACATTTCACTGGTCA  429739
Sbj01  20079   ............................................................  20020
Sbj02  311959  ............................................................  311900
Sbj03  37848   ............................................................  37789
Sbj04  67474   .C..........................................................  67533
Sbj05  128467  .C..........................................................  128526
Sbj06  136800  ....................................C.......................  136859
Sbj07  219647  ............................................................  219588


Query  429740  TGAGCTTGGTGAGAGAAGCGATACGAATGACCGAATCAAGCTGTGGACGAACATTATTGC  429799
Sbj01  20019   ............................................................  19960
Sbj02  311899  ............................................................  311840
Sbj03  37788   ............................................................T.  37729
Sbj04  67534   ..............................G.............................  67593
Sbj05  128527  ..............................G.............................  128586
Sbj06  136860  .T........................A...............................  136919
Sbj07  219587  .T..........................................................  219528

Query  429800  CCGGTCTGGTGTCACCGAAACTACGAAACACGCGTTGATTGCCGTCGATCACCACCAGCG  429859
Sbj01  19959   ............................................................  19900
Sbj02  311839  ............................................................  311780
Sbj03  37728   ............................................................  37669
Sbj04  67594   ............................................................  67653
Sbj05  128587  ............................................................  128646
Sbj06  136920  ..........T.....................................T..........  136979
Sbj07  219527  ..........T..................................................  219468
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Query  429860  CCATGCCCGTGGCGCCGCTACCATAAAAGATATGCTCGGCATAACGATCAACGATATCAG  429919
Sbj01  19899   ............................................................  19840
Sbj02  311779  ............A...............................................  311720
Sbj03  37668   ............................................................  37609
Sbj04  67654   ............................................................  67713
Sbj05  128647  ............................................................  128706
Sbj06  136980  ........................................G...................  137039
Sbj07  219467  ............................................................  219408

Query  429920  AGGCAAAAACCGGATCGGCAATCGGCTGAGCCGCCTGGACAGAAGTAAGTGATGCCGCAA  429979
Sbj01  19839   ............................................................  19780
Sbj02  311719  ............................................................  311660
Sbj03  37608   ............................................................  37549
Sbj04  67714   ............................................................  67773
Sbj05  128707  ............................................................  128766
Sbj06  137040  ............................................................  137099
Sbj07  219407  ............................................................  219348

Query  429980  ACAGCATGGCAGTAAAAAGCAGACTACGTTTCAACGGTGATGTCCATAAGTGAAATAACG  430039
Sbj01  19779   ............................................................  19720
Sbj02  311659  ............................................................  311600
Sbj03  37548   ............................................................A  37489
Sbj04  67774   ............................................................  67833
Sbj05  128767  ............................................................  128826
Sbj06  137100  ....TG........................................A..........  137159
Sbj07  219347  .....G..A.....................................A........  219288

Query  430040  GTGGTTATGCGTATTTATACTACCCGGCGGCACATTGCAAAACGTTAAAGAGAAAAAAGT  430099
Sbj01  19719   ............................................................  19660
Sbj02  311599  ............................................................  311540
Sbj03  37488   ...A........................................................  37429
Sbj04  67834   ............................................................  67893
Sbj05  128827  ............................................................  128886
Sbj06  137160  .A.....................................GT.................  137219
Sbj07  219287  ............................................................  219228
```

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Query  430100  G  430100
Sbj01  19659   .  19659
Sbj02  311539  .  311539
Sbj03  37428   .  37428
Sbj04  67894   .  67894
Sbj05  128887  .  128887
Sbj06  137220  .  137220
Sbj07  219227  .  219227
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Appendix 05. Prediction of virulence factors from the MDR isolates compared to the reference strains Enteritidis strain P125109 and Typhimurium LT2 according to VFDB.

VFclass	Virulence factors	Related genes	Salmonella Monophasic 725/16(Prediction)	Salmonella Infantis 14/05(Prediction)	Salmonella Enteritidis 520/08(Prediction)	S.enterica subsp. enterica serovar Enteritidis str. P125109	S.enterica subsp. enterica serovar Typhimurium str. LT2	
				draft (draft)	draft (draft)	chromosome (NC_011294)	chromosome (NC_003197)	pSLT (NC_003277)
Capsule	Vi antigen	tviA	-	-	-	-	-	-
		tviB	-	-	-	-	-	-
		tviC	-	-	-	-	-	-
		tviD	-	-	-	-	-	-
		tviE	-	-	-	-	-	-
		vexA	-	-	-	-	-	-
		vexB	-	-	-	-	-	-
		vexC	-	-	-	-	-	-
		vexD	-	-	-	-	-	-
		vexE	-	-	-	-	-	-
Fimbrial adherence determinants	Agf/Csg	csgA	orf00259	orf00887	LOC00710	SEN1904	STM1144	-
		csgB	orf00260	orf00886	LOC00714	SEN1905	STM1143	-
		csgC	orf00258	orf00888	LOC00707	SEN1903	STM1145	-

		csgD	orf00263	orf00885	-	SEN1906	STM1142	-
		csgE	orf00264	orf00884	LOC00709	SEN1907	STM1141	-
		csgF	orf00265	orf00883	LOC00716	SEN1908	STM1140	-
		csgG	orf00266	orf00882	LOC00711	SEN1909	STM1139	-
	Bcf	bcfA	orf01730	orf02933	LOC04316	SEN0020	STM0021	-
		bcfB	orf01731	orf02932	LOC04279	SEN0021	STM0022	-
		bcfC	orf04013	orf02931	-	SEN0022	STM0023	-
		bcfD	orf04014	orf02930	LOC04283	SEN0023	STM0024	-
		bcfE	orf04015	orf02929	LOC04284	SEN0024	STM0025	-
		bcfF	orf04016	orf02928	LOC04286	SEN0025	STM0026	-
		bcfG	orf04017	orf02927	LOC04210	SEN0026	STM0027	-
	Fim	fimA	orf03139	orf03228	LOC05359	SEN0524	STM0543	-
		fimC	orf03137	orf03230	LOC01910	SEN0526	STM0545	-
		fimD	orf03136	orf03231	LOC01909	SEN0527	STM0546	-
		fimF	orf03134	orf03233	LOC01903	SEN0529	STM0548	-
		fimH	orf03135	orf03232	LOC01906	SEN0528	STM0547	-
		fimI	orf03138	orf03229	-	SEN0525	STM0544	-
		fimW	orf03130	orf03238	LOC01904	SEN0533	STM0552	-
		fimY	-	orf03235	LOC01907	SEN0531	STM0550	-
		fimZ	orf03133	orf03234	LOC01905	SEN0530	STM0549	-

	Lpf	lpfA	orf01870	orf02059	LOC05492	SEN3463	STM3640	-
		lpfB	orf01869	orf02058	LOC05490	SEN3462	STM3639	-
		lpfC	orf01868	orf02057	LOC05498	SEN3461	STM3638	-
		lpfD	orf01867	orf02056	LOC05497	SEN3460	STM3637	-
		lpfE	orf01866	orf02055	LOC05493	SEN3459	STM3636	-
	Pef	pefA	-	-	LOC05732	-	-	PSLT018
		pefB	-	-	LOC05746	-	-	PSLT019
		pefC	-	-	LOC05741	-	-	PSLT017
		pefD	-	-	LOC05743	-	-	PSLT016
	Peg	pegA	-	-	LOC05141	SEN2145B	-	-
		pegB	-	-	LOC06211	SEN2145A	-	-
		pegC	-	-	LOC05142	SEN2145	-	-
		pegD	-	-	LOC05143	SEN2144A	-	-
	Saf	safA	-	-	LOC06257	SEN0281	STM0299	-
		safB	orf01025	orf02731	-	SEN0282	STM0300	-
		safC	orf01023	orf02730	LOC02191	SEN0283	STM0301	-
		safD	orf01022	orf02729	LOC02186	SEN0284	STM0302	-
	Sef	sefA	-	-	LOC06288	SEN4247	-	-
		sefB	-	-	-	SEN4248	-	-
		sefC	-	-	LOC05674	SEN4249	-	-

		sefD	-	-	LOC06287	SEN4250	-	-
	Sta	staA	-	-	-	-	-	-
		staB	-	-	-	-	-	-
		staC	-	-	-	-	-	-
		staD	-	-	-	-	-	-
		staE	-	-	-	-	-	-
		staF	-	-	-	-	-	-
		staG	-	-	-	-	-	-
	Stb	stbA	orf04482	orf02683	LOC02158	SEN0323	STM0340	-
		stbB	orf04481	orf02684	LOC02115	SEN0322	STM0339	-
		stbC	orf04480	orf02685	LOC02123	SEN0321	STM0338	-
		stbD	-	orf02686	LOC02127	SEN0320	STM0337	-
		stbE	orf04784	orf02687	LOC02126	SEN0319	STM0336	-
	Stc	stcA	orf01374	-	-	-	STM2152	-
		stcB	orf03392	orf01445	-	-	STM2151	-
		stcC	orf03393	orf01444	-	-	STM2150	-
		stcD	orf03394	-	-	-	STM2149	-
	Std	stdA	orf01479	orf01954	LOC01357	SEN2873	STM3029.S	-
		stdB	orf01480	orf01955	LOC05241	SEN2872	STM3028	-
		stdC	orf01481	orf01956	LOC01354	SEN2871	STM3027	-

	Ste	steA	-	-	-	SEN2794	-	-
		steB	-	-	LOC01438	SEN2795	-	-
		steC	-	-	LOC01440	SEN2796	-	-
		steD	-	-	LOC01590	SEN2797	-	-
		steE	-	-	LOC01441	SEN2798	-	-
		steF	-	-	LOC06234	SEN2799	-	-
	Stf	stfA	orf04764	orf03648	LOC05944	SEN0200	STM0195	-
		stfC	orf04765	orf03647	LOC05943	SEN0201	STM0196	-
		stfD	-	orf03646	LOC06349	SEN0202	STM0197	-
		stfE	orf01220	orf03645	LOC05941	SEN0203	STM0198	-
		stfF	orf01221	orf03644	LOC05938	SEN0204	STM0199	-
		stfG	orf01222	orf03643	LOC05939	SEN0205	STM0200	-
	Stg	stgA	-	-	-	-	-	-
		stgB	-	-	-	-	-	-
		stgC	-	-	-	-	-	-
		stgD	-	-	-	-	-	-
	Sth	sthA	orf01702	orf03508	LOC04245	SEN4351	STM4595	-
		sthB	orf01701	orf03509	LOC04249	SEN4350	STM4594	-
		sthC	orf01700	orf03510	-	SEN4349	STM4593	-
		sthD	orf01699	orf03511	LOC04248	SEN4348	STM4592	-

		sthE	orf01698	orf03512	LOC06026	SEN4347	STM4591	-
	Sti	stiA	orf01534	orf03670	LOC05951	SEN0182	STM0177	-
		stiB	orf01535	orf03671	LOC05959	SEN0181	STM0176	-
		stiC	orf01536	orf03672	LOC05952	SEN0180	STM0175	-
		stiH	orf01537	orf03673	LOC05956	SEN0179	STM0174	-
	Stj	Undetermined	orf01419	-	-	-	STM4571	-
		Undetermined	orf04316	-	-	-	STM4574	-
		Undetermined	-	-	-	-	STM4575	-
		stjB	orf01420	-	-	-	STM4572	-
		stjC	orf04315	-	-	-	STM4573	-
	Stk	stkA	-	-	-	-	-	-
		stkB	-	-	-	-	-	-
		stkC	-	-	-	-	-	-
		stkD	-	-	-	-	-	-
		stkE	-	-	-	-	-	-
		stkF	-	-	-	-	-	-
		stkG	-	-	-	-	-	-
	Tcf	tcfA	-	-	-	-	-	-
		tcfB	-	orf02722	-	-	-	-

		tcfC	-	orf02721	-	-	-	-
		tcfD	-	orf02720	-	-	-	-
Macrophage inducible genes	Mig-14	mig-14	orf03591	orf04311	LOC01634	SEN2626	STM2782	-
	Mig-5	mig-5	-	-	LOC05720	-	-	PSLT046
Magnesium uptake	Mg2+ transport	mgtB	orf04768	orf02187	LOC02569	SEN3585	STM3763	-
		mgtC	orf04930	orf02188	LOC02492	SEN3586	STM3764	-
Nonfimbrial adherence determinants	MisL	misL	orf04488	orf02182	LOC05525	SEN3580	STM3757	-
	RatB	ratB	-	orf00761	LOC05544	SEN2494*	STM2514	-
	ShdA	shdA	orf01969	orf00759	-	SEN2493	STM2513	-
	SinH	sinH	orf00935	orf00765	LOC06272	SEN2497*	STM2517	-
Regulation	PhoPQ	phoP	orf02222	orf00976	LOC00619	SEN1818	STM1231	-
		phoQ	orf02223	orf00975	LOC00736	SEN1819	STM1230	-
Secretion system	TTSS (SPI-1 encode)	hilA	orf03892	orf03297	LOC01506	SEN2718	STM2876	-
		hilC	orf03883	orf03288	LOC01518	SEN2709	STM2867	-
		hilD	orf03891	orf03296	LOC01507	SEN2717	STM2875	-
		iacP	orf04650	orf03302	LOC01509	SEN2722	STM2881	-
		iagB	orf03893	orf03298	LOC01512	SEN2719	STM2877	-
		invA	orf02754	orf03317	LOC01484	SEN2737	STM2896	-
		invB	orf02755	orf03316	LOC01489	SEN2736	STM2895	-
		invC	orf02756	orf03315	LOC01490	SEN2735	STM2894	-

		invE	orf02753	orf03318	LOC01487	SEN2738	STM2897	-
		invF	orf02751	orf03320	LOC01483	SEN2740	STM2899	-
		invG	orf02752	orf03319	LOC01482	SEN2739	STM2898	-
		invH	orf02749	orf03321	LOC01598	SEN2741	STM2900	-
		invI	orf02757	orf03314	LOC01495	SEN2734	STM2893	-
		invJ	orf02758	orf03313	LOC01493	SEN2733	STM2892	-
		orgA	orf03886	orf03291	LOC01519	SEN2712	STM2870	-
		orgB	orf03885	orf03290	-	SEN2711	STM2869	-
		orgC	orf03884	orf03289	LOC01228	SEN2710	STM2868	-
		prgH	orf03890	orf03295	LOC01514	SEN2716	STM2874	-
		prgI	orf03889	orf03294	LOC01511	SEN2715	STM2873	-
		prgJ	orf03888	orf03293	LOC01501	SEN2714	STM2872	-
		prgK	orf03887	orf03292	LOC01513	SEN2713	STM2871	-
		sicA	orf02764	orf03307	LOC01505	SEN2727	STM2886	-
		sicP	orf03895	orf03300	LOC01510	SEN2721	STM2879	-
		sipD	orf04648	orf03304	LOC05278	SEN2724	STM2883	-
		spaO	orf02759	orf03312	LOC01492	SEN2732	STM2891	-
		spaP	orf02760	orf03311	LOC01599	SEN2731	STM2890	-
		spaQ	orf02761	orf03310	LOC01499	SEN2730	STM2889	-
		spaR	orf02762	orf03309	LOC01600	SEN2729	STM2888	-

		spaS	orf02763	orf03308	LOC01502	SEN2728	STM2887	-
		sprB	orf05069	orf03287	LOC01517	SEN2708	STM2866	-
	TTSS (SPI-2 encode)	ssaC	orf00020	orf01143	LOC00463	SEN1651	STM1394	-
		ssaD	orf00019	orf01144	LOC00456	SEN1650	STM1395	-
		ssaE	orf00018	orf01145	LOC00458	SEN1649	STM1396	-
		ssaG	orf00009	orf01154	LOC00434	SEN1639	STM1406	-
		ssaH	orf00008	orf01155	LOC00439	SEN1638	STM1407	-
		ssaI	orf00007	orf01156	LOC00435	SEN1637	STM1408	-
		ssaJ	orf00006	orf01157	LOC00438	SEN1636	STM1409	-
		ssaK	orf00004	orf01159	LOC00430	SEN1634	STM1411	-
		ssaL	orf00003	orf01160	LOC00427	SEN1633	STM1412	-
		ssaM	orf00002	orf01161	LOC00432	SEN1632	STM1413	-
		ssaN	orf05040	orf01163	LOC00448	SEN1630	STM1415	-
		ssaO	orf05039	orf01164	LOC00425	SEN1629	STM1416	-
		ssaP	orf05038	orf01165	LOC00426	SEN1628	STM1417	-
		ssaQ	-	orf01166	LOC00428	SEN1627	STM1418	-
		ssaR	orf00550	orf01167	LOC00424	SEN1626	STM1419	-
		ssaS	orf00551	-	LOC00420	SEN1625	STM1420	-
		ssaT	orf00552	orf01169	LOC00450	SEN1624	STM1421	-
		ssaU	orf00553	orf01170	LOC00423	SEN1623	STM1422	-

		ssaV	orf00001	orf01162	LOC00433	SEN1631	STM1414	-
		sscA	orf00016	orf01147	LOC00443	SEN1646	STM1399	-
		sscB	orf00012	orf01151	LOC00440	SEN1642	STM1403	-
		sseA	-	-	LOC00446	SEN1648	STM1397	-
		sseB	orf00017	orf01146	LOC00445	SEN1647	STM1398	-
		sseC	orf00015	orf01148	LOC00449	SEN1645	STM1400	-
		sseD	orf00014	orf01149	LOC00444	SEN1644	STM1401	-
		sseE	orf00013	orf01150	LOC00441	SEN1643	STM1402	-
		ssrA	orf00022	orf01142	LOC00459	SEN1653	STM1392	-
		ssrB	orf00023	orf01141	LOC00462	SEN1654	STM1391	-
	TTSS effectors translocated via both systems	slrP	orf01844	orf04200	LOC05761; LOC06315; LOC06319	SEN0745*	STM0800	-
		sspH1	-	-	-	-		-
	TTSS-1 translocated effectors	avrA	orf05082	orf03286	LOC06230	SEN2707	STM2865	-
		sipA	orf04649	orf03303	LOC01504	SEN2723	STM2882	-
		sipB	orf04646	orf03306	LOC01498	SEN2726	STM2885	-
		sipC	orf04647	orf03305	LOC01226	SEN2725	STM2884	-
		sopA	orf02253	orf01360	LOC05127	SEN2065	STM2066	-
		sopB/sigD	orf00319	orf04145	LOC03631	SEN0955	STM1091	-
		sopD	orf04249	orf03368	LOC01443	SEN2784	STM2945	-

		sopE2	orf04848	orf00110	LOC05866	SEN1182	STM1855	-
		sopE	-	-	LOC03792	SEN1155	-	-
		sptP	orf03894	orf03299	LOC01503	SEN2720	STM2878	-
	TTSS-2 translocated effectors	gogB	orf03623	-	-	-	STM2584	-
		pipB2	orf03588	orf04314	LOC01648	SEN2624	STM2780	-
		pipB	orf00322	orf04142	LOC03632	SEN0952	STM1088	-
		sifA	orf02230	orf00968	LOC00628	SEN1825	STM1224	-
		sifB	orf00248	orf00380	LOC00227	SEN1454	STM1602	-
		sopD2	-	-	LOC05796	SEN0876	-	-
		spiC/ssaB	orf00021	-	LOC00460	SEN1652	STM1393	-
		sseF	orf00011	orf01152	LOC00451	SEN1641	STM1404	-
		sseG	orf00010	-	LOC00455	SEN1640	STM1405	-
		sseI/srfH	orf00360	-	LOC05808	SEN0916	STM1051	-
		sseJ	orf00840	orf00349	LOC04869	SEN1422	STM1631	-
		sseK1	orf03607	orf04459	LOC06372	SEN3941	STM4157	-
		sseK2	orf03410	orf01428	LOC05033	SEN1920	STM2137	-
		sseL	orf01628	orf00526	LOC02995	SEN2269	STM2287	-
		sspH2	orf02269	orf01530	LOC06214	SEN2224	STM2241	-
Serum resistance	Rck	rck	-	-	LOC05679	-	-	PSLT009
Spv locus	Spv	spvA	-	-	LOC05727	-	-	PSLT040

		spvB	-	-	LOC05726	-	-	PSLT039
		spvC	-	-	LOC05731	-	-	PSLT038
		spvD	-	-	LOC05728	-	-	PSLT037
		spvR	-	-	LOC06298	-	-	PSLT041
Stress adaptation	SodCI	sodCI	orf04295	-	LOC05889	SEN1149	STM1044	-
Toxin	Typhoid toxin	cdtB	-	-	-	-	-	-
		pltA	-	-	-	-	-	-
		pltB	-	-	-	-	-	-
Others	O-antigen(Yersinia)		-	orf01379	-	-	-	-
Immune evasion	LPS glucosylation(Shigella)	gtrA	orf03475	-	-	-	-	-
Invasion	Invasin A(Yersinia)		orf05094	-	-	-	-	-
	Invasion of brain endothelial cells (Ibes)(Escherichia)	ibeB	orf03238	-	-	-	-	-

Appendix 06. Prediction of virulence factors from the Typhi isolates compared to the reference strains Typhi Ty2 and Typhimurium LT2 according to VFDB.

Virulence factors	Related genes	Salmonella Typhi 13/11 (Prediction)	Salmonella Typhi 303/06 (Prediction)	Salmonella Typhi 328/06 (Prediction)	Salmonella Typhi 385/06 (Prediction)	S.enterica subsp. enterica serovar Typhi str. Ty2	S.enterica subsp. enterica serovar Typhimurium str. LT2	
		draft (draft)	draft (draft)	draft (draft)	draft (draft)	chromosome (NC_004631)	chromosome (NC_003197)	pSLT (NC_003277)
Vi antigen	tviA	-	LOC03906	LOC03833	-	t4353	-	-
	tviB	orf04390	LOC03908	LOC03841	orf03335	t4352	-	-
	tviC	orf04389	LOC03912	LOC03792	orf03336	t4351	-	-
	tviD	orf04388	LOC03907	LOC03826	orf03337	t4350	-	-
	tviE	orf04387	LOC03905	LOC03827	orf03338	t4349	-	-
	vexA	orf04386	LOC03910	LOC03831	orf03339	t4348	-	-
	vexB	orf04385	LOC03874	LOC03817	orf03340	t4347	-	-
	vexC	orf04384	LOC03903	LOC03840	orf03341	t4346	-	-
	vexD	orf04383	LOC03902	LOC03825	orf03343	t4345	-	-
	vexE	orf04382	LOC03904	LOC03824	orf03344	t4344	-	-
Agf/Csg	csgA	orf03804	LOC04729	LOC04655	orf03844	t1776	STM1144	-
	csgB	orf03803	LOC04730	LOC04653	orf03845	t1777	STM1143	-

	csgC	orf03805	LOC04731	LOC04654	-	t1775	STM1145	-
	csgD	orf03802	LOC04723	LOC04657	orf03848	t1778	STM1142	-
	csgE	orf03801	LOC04676	LOC04658	orf03849	t1779	STM1141	-
	csgF	orf03800	LOC04675	LOC04661	orf03850	t1780	STM1140	-
	csgG	orf03799	LOC04725	LOC04660	orf03851	t1781	STM1139	-
Bcf	bcfA	orf01167	LOC01499	LOC02121	orf01546	t0022	STM0021	-
	bcfB	orf01168	LOC01500	LOC02045	orf01545	t0023	STM0022	-
	bcfC	orf01169; orf01170; orf01172	-	-	orf01541; orf01543; orf01544	t0024*	STM0023	-
	bcfD	orf01173	LOC01498	LOC02127	orf01540	t0025	STM0024	-
	bcfE	orf01174	LOC01501	LOC02129	orf01539	t0026	STM0025	-
	bcfF	orf01175	LOC01493	LOC02130	orf01538	t0027	STM0026	-
	bcfG	orf01176	LOC01497	LOC02131	orf01537	t0028	STM0027	-
Fim	fimA	orf02890	LOC03180	LOC03341	orf02888	t2320	STM0543	-
	fimC	orf02888	LOC03175	LOC03345	orf02890	t2318	STM0545	-
	fimD	orf02887	LOC03182	LOC03346	orf02891	t2317	STM0546	-
	fimF	orf02885	LOC03185	LOC03350	orf02893	t2315	STM0548	-
	fimH	orf02886	LOC03184	LOC03348	orf02892	t2316	STM0547	-
	fimI	orf02889	-	-	orf02889	t2319	STM0544	-
	fimW	orf02880	LOC03177	LOC03413	orf02898	t2311	STM0552	-

	fimY	orf02883	LOC03176	LOC03349	-	t2313	STM0550	-
	fimZ	orf02884	LOC03181	LOC03336	orf02894	t2314	STM0549	-
Lpf	lpfA	-	-	-	-	-	STM3640	-
	lpfB	-	-	-	-	-	STM3639	-
	lpfC	orf03897	-	-	orf04028	-	STM3638	-
	lpfD	-	-	-	-	-	STM3637	-
	lpfE	-	-	-	-	-	STM3636	-
Pef	pefA	-	-	-	-	-	-	PSLT018
	pefB	-	-	-	-	-	-	PSLT019
	pefC	-	-	-	-	-	-	PSLT017
	pefD	-	-	-	-	-	-	PSLT016
Peg	pegA	-	-	-	-	-	-	-
	pegB	-	-	-	-	-	-	-
	pegC	-	-	-	-	-	-	-
	pegD	-	-	-	-	-	-	-
Saf	safA	-	LOC02934	LOC03017	-	t2561	STM0299	-
	safB	orf04208	LOC02833	LOC03014	orf02552	t2559	STM0300	-
	safC	orf04209	LOC02837	LOC03012	orf02553	t2558	STM0301	-
	safD	orf04210	LOC02836	LOC03011	orf02554	t2557	STM0302	-
Sef	sefA	-	-	-	-	t4533*	-	-

	sefB	-	LOC01629	LOC01974	-	t4534	-	-
	sefC	orf01023	LOC06018	LOC06048	orf01678	t4536	-	-
	sefD	-	-	-	-	t4535*	-	-
Sta	staA	orf02233	LOC02136	LOC02509	-	t0190	-	-
	staB	orf02234	LOC02131	LOC02516	orf02011	t0189	-	-
	staC	orf02235	LOC02135	LOC02514	orf02012	t0188	-	-
	staD	orf02236	LOC02133	LOC02515	orf02013	t0187	-	-
	staE	orf02237	LOC02129	LOC02518	-	t0186	-	-
	staF	orf02238	LOC02130	LOC02519	orf02014	t0185	-	-
	staG	orf02239	LOC07063	LOC06776	orf02015	t0184	-	-
Stb	stbA	orf03712	LOC02869	LOC02975	orf02591	t2521	STM0340	-
	stbB	orf03713	LOC02867	LOC02974	orf02590	t2522	STM0339	-
	stbC	orf03714	LOC02862	LOC02973	orf02589	t2523*	STM0338	-
	stbD	orf03715	LOC02866	LOC02979	orf02588	t2524	STM0337	-
	stbE	orf03716	LOC02859	LOC03053	orf02587	t2525	STM0336	-
Stc	stcA	orf00091	LOC00320	LOC00121	orf00093	t0704	STM2152	-
	stcB	orf00092	LOC00117	LOC00116	orf00094	t0705	STM2151	-
	stcC	orf00093	LOC00120	LOC00327	orf00095	t0706*	STM2150	-
	stcD	orf00094	LOC00168	LOC00163	orf00096	t0707	STM2149	-
Std	stdA	orf04047	LOC04895	LOC04983	orf04149	t2940	STM3029.S	-

	stdB	orf04046	LOC04900	LOC04984	orf04148	t2939	STM3028	-
	stdC	orf04045	LOC04894	LOC04979	orf04147	t2938	STM3027	-
Ste	steA	orf01539	-	-	orf01450	t2856*	-	-
	steB	orf01538	LOC02367	LOC02826	orf01451	t2857	-	-
	steC	orf01537	LOC02430	LOC02829	orf01452	t2858	-	-
	steD	orf01536	LOC02209	LOC02828	orf01453	t2859	-	-
	steE	orf01535	LOC02431	LOC02833	orf01454	t2860	-	-
	steF	orf01534	LOC02371	LOC02831	orf01455	t2861	-	-
Stf	stfA	-	-	-	-	-	STM0195	-
	stfC	-	-	-	-	-	STM0196	-
	stfD	-	-	-	-	-	STM0197	-
	stfE	-	-	-	-	-	STM0198	-
	stfF	-	-	-	-	-	STM0199	-
	stfG	-	-	-	-	-	STM0200	-
Stg	stgA	orf03900	LOC04863	LOC04840	orf04031	t3659	-	-
	stgB	orf03899	LOC04816	LOC04836	orf04030	t3660	-	-
	stgC	-	-	-	-	t3661*	-	-
	stgD	orf03896	LOC04819	LOC04837	orf04027	t3662	-	-
Sth	sthA	orf01137	LOC01531	LOC02044	orf01573	t4634	STM4595	-
	sthB	orf01136	LOC01439	LOC02092	orf01574	t4633	STM4594	-

	sthC	orf01135	-	-	orf01575	t4632*	STM4593	-
	sthD	orf01134	LOC01530	LOC02089	orf01576	t4631	STM4592	-
	sthE	orf01132	-	-	orf01578	t4630*	STM4591	-
Sti	stiA	-	-	-	-	-	STM0177	-
	stiB	-	-	-	-	-	STM0176	-
	stiC	-	-	-	-	-	STM0175	-
	stiH	-	-	-	-	-	STM0174	-
Stj	Undetermined	-	-	-	-	-	STM4571	-
	Undetermined	-	-	-	-	-	STM4574	-
	Undetermined	-	-	-	-	-	STM4575	-
	stjB	-	-	-	-	-	STM4572	-
	stjC	-	-	-	-	-	STM4573	-
Stk	stkA	-	-	-	-	-	-	-
	stkB	-	-	-	-	-	-	-
	stkC	-	-	-	-	-	-	-
	stkD	-	-	-	-	-	-	-
	stkE	-	-	-	-	-	-	-
	stkF	-	-	-	-	-	-	-
	stkG	-	-	-	-	-	-	-
Tcf	tcfA	orf04218	LOC02841	LOC03003	-	t2550	-	-

	tcfB	orf04219	LOC02834	LOC03005	orf02561	t2549	-	-
	tcfC	orf03741	LOC02843	LOC03001	orf02562	t2548	-	-
	tcfD	orf03740	LOC02845	LOC03000	orf02563	t2547	-	-
Mig-14	mig-14	orf01728	LOC04426	LOC04298	orf01266	t2676	STM2782	-
Mig-5	mig-5	-	-	-	-	-	-	PSLT046
Mg2+ transport	mgtB	orf00595	LOC01021	LOC01023	orf00555	t3755	STM3763	-
	mgtC	orf00593	LOC01022	LOC01026	orf00554	t3754	STM3764	-
MisL	misL	orf00602; orf00603	-	-	orf00562; orf00563	t3760*	STM3757	-
RatB	ratB	orf02429; orf02431	-	-	orf02404; orf02406	t0342*	STM2514	-
ShdA	shdA	orf02425	-	-	orf02400	t0344*	STM2513	-
SinH	sinH	orf02435	-	-	orf02410	t0339*	STM2517	-
PhoPQ	phoP	orf04335	LOC05176	LOC05121	orf04331	t1689	STM1231	-
	phoQ	orf04334	LOC05179	LOC05124	orf04332	t1690	STM1230	-
TTSS (SPI-1 encode)	hilA	orf01622	LOC02202	LOC02748	orf01368	t2778	STM2876	-
	hilC	orf01634	LOC02272	LOC02887	orf01360	t2769	STM2867	-
	hilD	orf01624	LOC02277	LOC02744	orf01367	t2777	STM2875	-
	iacP	orf01616	LOC02287	LOC02751	orf01373	t2783	STM2881	-
	iagB	orf01621	LOC02283	LOC02749	orf01369	t2779	STM2877	-
	invA	orf01601	LOC02305	LOC02765	orf01388	t2798	STM2896	-

	invB	orf01602	LOC02302	LOC02767	orf01387	t2797	STM2895	-
	invC	orf01603	LOC02204	LOC02890	orf01386	t2796	STM2894	-
	invE	orf01600	LOC02303	LOC02768	orf01389	t2799	STM2897	-
	invF	orf01598	LOC02299	LOC02674	orf01391	t2801	STM2899	-
	invG	orf01599	LOC02306	LOC02770	orf01390	t2800	STM2898	-
	invH	orf01597	LOC02304	LOC02774	orf01392	t2802	STM2900	-
	invI	orf01604	LOC02301	LOC02766	orf01385	t2795	STM2893	-
	invJ	orf01605	LOC02300	LOC02763	orf01384	t2794	STM2892	-
	orgA	orf01630	LOC02270	LOC02743	-	t2772	STM2870	-
	orgB	orf01631	LOC02273	LOC02739	orf01362	t2771	STM2869	-
	orgC	orf01632	LOC02423	LOC02740	orf01361	t2770	STM2868	-
	prgH	orf01626	LOC02279	LOC02671	orf01366	t2776	STM2874	-
	prgI	orf01627	LOC02274	LOC02746	orf01365	t2775	STM2873	-
	prgJ	orf01628	LOC02276	LOC02742	orf01364	t2774	STM2872	-
	prgK	orf01629	LOC02261	LOC02741	orf01363	t2773	STM2871	-
	sicA	orf01611	LOC02289	LOC02757	orf01378	t2788	STM2886	-
	sicP	orf01618	LOC02280	LOC02672	orf01371	t2781	STM2879	-
	sipD	orf01614	LOC02285	LOC02754	orf01375	t2785	STM2883	-
	spaO	orf01606	LOC02297	LOC02761	orf01383	t2793	STM2891	-
	spaP	orf01607	LOC02288	LOC02673	orf01382	t2792	STM2890	-

	spaQ	orf01608	LOC02293	LOC02759	orf01381	t2791	STM2889	-
	spaR	orf01609	LOC02292	LOC02755	orf01380	t2790	STM2888	-
	spaS	orf01610	LOC02291	LOC02758	orf01379	t2789	STM2887	-
	sprB	orf01635	LOC02271	LOC02738	orf01359	t2768	STM2866	-
TTSS (SPI-2 encode)	ssaC	orf03358	LOC03784	LOC03711	orf02289	t1262	STM1394	-
	ssaD	orf03357	LOC03783	LOC03709	orf02288	t1263	STM1395	-
	ssaE	orf03356	LOC03781	LOC03707	orf02287	t1264	STM1396	-
	ssaG	orf03347	LOC03775	LOC03692	orf02278	t1274	STM1406	-
	ssaH	orf03346	LOC03774	LOC03687	orf02277	t1275	STM1407	-
	ssaI	-	LOC03771	LOC03690	-	t1276	STM1408	-
	ssaJ	orf03344	LOC03769	LOC03688	orf02275	t1277	STM1409	-
	ssaK	orf03342	LOC03687	LOC03685	orf02273	t1279	STM1411	-
	ssaL	orf03341	LOC03765	LOC03681	orf02272	t1280	STM1412	-
	ssaM	orf03340	LOC03770	LOC03680	-	t1281	STM1413	-
	ssaN	orf03338	LOC03761	LOC03675	orf02270	t1283	STM1415	-
	ssaO	orf03337	LOC03760	LOC03604	orf02269	t1284	STM1416	-
	ssaP	orf03336	LOC03759	LOC03678	-	t1285	STM1417	-
	ssaQ	orf03335	LOC03758	LOC03603	orf02268	t1286	STM1418	-
	ssaR	orf03334	LOC03756	LOC03673	orf02267	t1287	STM1419	-
	ssaS	-	LOC03684	LOC03674	-	t1288	STM1420	-

	ssaT	orf03332	LOC03750	LOC03677	orf02266	t1289	STM1421	-
	ssaU	orf03331	LOC03685	LOC03670	orf02265	t1290	STM1422	-
	ssaV	orf03339	LOC03764	LOC03679	orf02271	t1282	STM1414	-
	sscA	orf03354	LOC03782	LOC03696	orf02285	t1267	STM1399	-
	sscB	orf03350	LOC03779	LOC03686	orf02281	t1271	STM1403	-
	sseA	-	LOC03780	LOC03695	-	t1265	STM1397	-
	sseB	orf03355	LOC03677	LOC03697	orf02286	t1266	STM1398	-
	sseC	orf03353	LOC03772	LOC03694	orf02284	t1268	STM1400	-
	sseD	orf03352	LOC03768	LOC03703	orf02283	t1269	STM1401	-
	sseE	orf03351	LOC03845	LOC03693	orf02282	t1270	STM1402	-
	ssrA	orf03359	LOC03786	LOC03708	orf02290	t1260	STM1392	-
	ssrB	orf03360	LOC03785	LOC03715	orf02291	t1259	STM1391	-
TTSS effectors translocated via both systems	slrP	orf02146	-	-	orf01792	t2087*	STM0800	-
	sspH1	-	-	-	-	-		-
TTSS-1 translocated effectors	avrA	-	-	-	-	-	STM2865	-
	sipA	orf01615	LOC02281	LOC02752	orf01374	t2784	STM2882	-
	sipB	orf01612	LOC02290	LOC02889	orf01377	t2787	STM2885	-
	sipC	orf01613	LOC02278	LOC02756	orf01376	t2786	STM2884	-
	sopA	orf00196; orf00198	-	-	orf00195	t0808*	STM2066	-
	sopB/sigD	orf03747	LOC04663	LOC04693	orf03905	t1828	STM1091	-

	sopD	orf01551	LOC02355	LOC02814	orf01437	t2846	STM2945	-
	sopE2	orf00445	-	-	orf00413	-	STM1855	-
	sopE	-	LOC01428	LOC01896	-	t4303	-	-
	sptP	orf01619; orf01620	LOC02282	LOC02888	orf01370	t2780	STM2878	-
TTSS-2 translocated effectors	gogB	-	-	-	-	-	STM2584	-
	pipB2	orf01731	LOC04380	LOC04304	orf01264	t2674	STM2780	-
	pipB	orf03744	LOC04664	LOC04687	orf03907	t1830	STM1088	-
	sifA	orf04327	LOC05170	LOC05116	orf04338	t1696	STM1224	-
	sifB	orf02576	LOC03010	LOC03173	orf02700	t1511	STM1602	-
	sopD2	-	-	-	-	t1962*	-	-
	spiC/ssaB	-	LOC03787	LOC03712	-	t1261	STM1393	-
	sseF	orf03349	LOC03777	LOC03689	orf02280	t1272	STM1404	-
	sseG	orf03348	LOC03767	LOC03683	orf02279	t1273	STM1405	-
	sseI/srfH	-	-	-	-	-	STM1051	-
	sseJ	-	-	-	-	t1534*	STM1631	-
	sseK1	-	-	-	-	-	STM4157	-
	sseK2	-	-	-	-	-	STM2137	-
	sseL	orf03107	LOC03313	LOC01112	orf03071	t0576	STM2287	-
	sspH2	orf00008	LOC00082	LOC00057	orf00007	t0623	STM2241	-
Rck	rck	-	-	-	-	-	-	PSLT009

Spv	spvA	-	-	-	-	-	-	PSLT040
	spvB	-	-	-	-	-	-	PSLT039
	spvC	-	-	-	-	-	-	PSLT038
	spvD	-	-	-	-	-	-	PSLT037
	spvR	-	-	-	-	-	-	PSLT041
SodCI	sodCI	-	-	-	-	-	STM1044	-
Typhoid toxin	cdtB	orf04717	LOC05458	LOC05423	orf04595	t1111	-	-
	pltA	orf04714	LOC06731	LOC06715	orf04593	t1108	-	-
	pltB	-	LOC05461	LOC05425	-	t1107	-	-
Invasin A(Yersinia)		orf00540; orf04995	-	-	-	-	-	-

Appendix 07. Prediction of virulence factors from the subspecies non-*enterica* isolates compared to the reference strains *S. arizonae* strain RSK2980 and Typhimurium LT2 according to VFDB.

VFclass	Virulence factors	Related genes	Salmonella diarizonae 08/16 (Prediction)	Salmonella diarizonae 30/10 (Prediction)	Salmonella salamae 644/10 (Prediction)	S.enterica subsp. arizonae serovar 62:z4,z23:-- str. RSK2980	S.enterica subsp. enterica serovar Typhimurium str. LT2	
			draft (draft)	draft (draft)	draft (draft)	chromosome (NC_010067)	chromosome (NC_003197)	pSLT (NC_003277)
Capsule	Vi antigen	tviA	-	-	-	-	-	-
		tviB	-	-	-	-	-	-
		tviC	-	-	-	-	-	-
		tviD	-	-	-	-	-	-
		tviE	-	-	-	-	-	-
		vexA	-	-	-	-	-	-
		vexB	-	-	-	-	-	-
		vexC	-	-	-	-	-	-
		vexD	-	-	-	-	-	-
		vexE	-	-	-	-	-	-
Fimbrial adherence determinants	Agf/Csg	csgA	LOC03327	orf03759	LOC03071	SARI_01854	STM1144	-
		csgB	LOC03323	orf03760	LOC03068	SARI_01855	STM1143	-
		csgC	LOC03326	orf03758	LOC03066	SARI_01853	STM1145	-
		csgD	LOC05431	orf03762	LOC05290	SARI_01856*	STM1142	-
		csgE	LOC03321	orf03763	LOC03065	-	STM1141	-
		csgF	LOC03318	orf03764	LOC03061	SARI_01857*	STM1140	-

		csgG	LOC03319	orf03765	LOC03057	SARI_01858*	STM1139	-
	Bcf	bcfA	LOC00222	orf01265	LOC00972	SARI_02971	STM0021	-
		bcfB	LOC00214	orf01264	LOC00853	SARI_02970	STM0022	-
		bcfC	LOC05357	orf01263; orf04582	-	SARI_02969	STM0023	-
		bcfD	LOC00223	orf01262	LOC00856	SARI_02968	STM0024	-
		bcfE	LOC03964	orf01261	LOC00854	SARI_02967	STM0025	-
		bcfF	LOC00217	orf01260	LOC00855	SARI_02966	STM0026	-
		bcfG	-	orf01259	LOC00857	SARI_02965	STM0027	-
	Fim	fimA	LOC04193	orf02342	LOC04353	SARI_02411	STM0543	-
		fimC	LOC00591	orf02344	LOC00562	SARI_02409	STM0545	-
		fimD	LOC00595; LOC04253	orf02144; orf02345	LOC00569	SARI_02408	STM0546	-
		fimF	LOC04191	orf02347	LOC04352	-	STM0548	-
		fimH	LOC00593	orf02346	LOC00564	SARI_02407	STM0547	-
		fimI	-	orf02343	-	SARI_02410	STM0544	-
		fimW	LOC00590	orf02352	LOC00567	-	STM0552	-
		fimY	LOC00813	-	LOC00566	-	STM0550	-
		fimZ	LOC00587	orf02348	LOC00565	-	STM0549	-
	Lpf	lpfA	-	-	-	-	STM3640	-
		lpfB	-	-	-	-	STM3639	-
		lpfC	LOC04989	orf01643; orf03235; orf04702	LOC04386	-	STM3638	-
		lpfD	-	-	-	-	STM3637	-
		lpfE	-	-	-	-	STM3636	-
	Pef	pefA	-	-	-	-	-	PSLT018

		pefB	-	-	-	-	-	PSLT019
		pefC	-	-	LOC06004	-	-	PSLT017
		pefD	-	-	-	-	-	PSLT016
	Peg	pegA	-	-	-	-	-	-
		pegB	LOC04709	orf02728	-	-	-	-
		pegC	LOC04705	orf02729	-	-	-	-
		pegD	-	-	-	-	-	-
	Saf	safA	-	-	-	-	STM0299	-
		safB	-	-	-	-	STM0300	-
		safC	-	-	-	-	STM0301	-
		safD	-	-	-	-	STM0302	-
	Sef	sefA	-	-	-	-	-	-
		sefB	-	-	-	-	-	-
		sefC	-	-	-	-	-	-
		sefD	-	-	-	-	-	-
	Sta	staA	-	-	LOC04506	-	-	-
		staB	-	-	LOC04504	-	-	-
		staC	-	-	LOC06000	-	-	-
		staD	-	-	LOC01007	-	-	-
		staE	-	-	LOC04501	-	-	-
		staF	-	-	LOC05998	-	-	-
		staG	-	-	-	-	-	-
	Stb	stbA	LOC00790	orf02165	-	-	STM0340	-
		stbB	LOC00755	orf02164	-	-	STM0339	-
		stbC	LOC04236	orf02163	-	-	STM0338	-
		stbD	LOC00754	orf02162	-	-	STM0337	-
		stbE	-	orf02161	-	-	STM0336	-
	Stc	stcA	LOC04701	orf02727	LOC05037	-	STM2152	-

		stcB	-	-	LOC02302	-	STM2151	-
		stcC	-	-	LOC02307	-	STM2150	-
		stcD	-	-	LOC02295	-	STM2149	-
	Std	stdA	LOC04413	orf00683; orf01041	-	-	STM3029.S	-
		stdB	LOC01028; LOC04875	orf00423; orf00684; orf01043; orf01684; orf04670	-	-	STM3028	-
		stdC	LOC01111; LOC04883	orf00424; orf00685; orf01044; orf01683; orf04669	-	-	STM3027	-
	Ste	steA	-	-	-	-	-	-
		steB	-	-	-	-	-	-
		steC	-	-	-	-	-	-
		steD	-	-	-	-	-	-
		steE	-	-	-	-	-	-
		steF	-	-	-	-	-	-
	Stf	stfA	-	-	LOC05118	-	STM0195	-
		stfC	-	-	LOC05124	-	STM0196	-
		stfD	-	-	LOC05122	-	STM0197	-
		stfE	-	-	LOC05120	-	STM0198	-
		stfF	-	-	LOC05119	-	STM0199	-
		stfG	-	-	LOC05115	-	STM0200	-
	Stg	stgA	-	-	-	-	-	-

		stgB	-	-	-	-	-	-
		stgC	-	-	-	-	-	-
		stgD	-	-	-	-	-	-
	Sth	sthA	-	-	LOC00830	-	STM4595	-
		sthB	-	-	LOC00956	-	STM4594	-
		sthC	-	-	LOC04443	-	STM4593	-
		sthD	-	-	LOC00967	-	STM4592	-
		sthE	-	-	LOC04437	-	STM4591	-
	Sti	stiA	-	-	-	-	STM0177	-
		stiB	-	-	-	-	STM0176	-
		stiC	-	-	-	-	STM0175	-
		stiH	-	-	-	-	STM0174	-
	Stj	Undetermined	-	-	-	-	STM4571	-
		Undetermined	-	-	-	-	STM4574	-
		Undetermined	-	-	-	-	STM4575	-
		stjB	-	-	-	-	STM4572	-
		stjC	-	-	-	-	STM4573	-
	Stk	stkA	-	-	-	-	-	-
		stkB	-	-	-	-	-	-
		stkC	-	-	-	-	-	-
		stkD	-	-	-	-	-	-
		stkE	-	-	-	-	-	-
		stkF	-	-	-	-	-	-
		stkG	-	-	-	-	-	-
	Tcf	tcfA	-	-	-	-	-	-
		tcfB	-	-	-	-	-	-
		tcfC	-	-	-	-	-	-
		tcfD	-	-	-	-	-	-

Macrophage inducible genes	Mig-14	mig-14	LOC00828	orf00963	LOC03817	SARI_00193	STM2782	-
	Mig-5	mig-5	-	-	-	-	-	PSLT046
Magnesium uptake	Mg2+ transport	mgtB	LOC01178	orf04474	LOC03538	SARI_03870	STM3763	-
		mgtC	LOC01177	orf04476	LOC03537	SARI_03869	STM3764	-
Nonfimbrial adherence determinants	MisL	misL	LOC04484	orf04469	misL	-	STM3757	-
	RatB	ratB	-	-	ratB	-	STM2514	-
	ShdA	shdA	-	-	shdA	-	STM2513	-
	SinH	sinH	-	-	sinH	-	STM2517	-
Regulation	PhoPQ	phoP	LOC03406	orf03673	phoP	SARI_01759	STM1231	-
		phoQ	LOC03404	orf03674	phoQ	SARI_01760	STM1230	-
Secretion system	TTSS (SPI-1 encode)	hilA	LOC00922	orf00867	LOC03356	SARI_00097	STM2876	-
		hilC	LOC04342	orf00877	-	SARI_00106	STM2867	-
		hilD	-	orf00869	-	SARI_00098	STM2875	-
		iacP	LOC01104	orf00862	LOC03342	SARI_00092	STM2881	-
		iagB	LOC00918	orf00866	LOC03358	SARI_00096	STM2877	-
		invA	LOC00934	orf00848	LOC03339	SARI_00077	STM2896	-
		invB	LOC00928	-	LOC03343	SARI_00078	STM2895	-
		invC	LOC01128	orf00849	LOC03340	SARI_00079	STM2894	-
		invE	LOC00937	orf00847	LOC03337	SARI_00076	STM2897	-
		invF	LOC00939	orf00845	LOC03333	SARI_00074	STM2899	-
		invG	-	orf00846	LOC03336	SARI_00075	STM2898	-
		invH	LOC00935	orf00844	LOC03331	SARI_00073	STM2900	-
		invI	LOC00933	orf00850	LOC03338	SARI_00080	STM2893	-
		invJ	LOC01127	orf00851	LOC03407	SARI_00081	STM2892	-
		orgA	LOC00910	orf00874	LOC03360	SARI_00103	STM2870	-
		orgB	-	orf00875	LOC03363	SARI_00104	STM2869	-
		orgC	LOC04340	orf00876	LOC03354	SARI_00105	STM2868	-
		prgH	LOC00920	orf00870	LOC03361	SARI_00099	STM2874	-

		prgI	LOC04343	orf00871	LOC05400	SARI_00100	STM2873	-
		prgJ	LOC00917	orf00872	LOC03362	SARI_00101	STM2872	-
		prgK	LOC00916	orf00873	LOC05397	SARI_00102	STM2871	-
		sicA	LOC00927	orf00857	LOC03350	SARI_00087	STM2886	-
		sicP	LOC04346	orf00864	LOC05392	SARI_00094	STM2879	-
		sipD	LOC04347	orf00860	LOC03351	SARI_00090	STM2883	-
		spaO	LOC00930	orf00852	LOC03347	SARI_00082	STM2891	-
		spaP	LOC00932	orf00853	LOC03345	SARI_00083	STM2890	-
		spaQ	LOC00929	orf00854	LOC03408	SARI_00084	STM2889	-
		spaR	LOC00931	orf00855	LOC03409	SARI_00085	STM2888	-
		spaS	LOC00926	orf00856	LOC03349	SARI_00086	STM2887	-
		sprB	LOC00915	orf00878	LOC05402	SARI_00107	STM2866	-
	TTSS (SPI-2 encode)	ssaC	LOC02599; LOC04287	orf03900	LOC01481	SARI_01587	STM1394	-
		ssaD	LOC02538	orf03899	LOC01604	SARI_01586	STM1395	-
		ssaE	LOC05062	orf03898	LOC04635	SARI_01585	STM1396	-
		ssaG	LOC02593	orf03888	LOC01474	SARI_01575	STM1406	-
		ssaH	LOC02598	orf03887	LOC01477	SARI_01574	STM1407	-
		ssaI	LOC05053	orf03886	LOC04628	SARI_01573	STM1408	-
		ssaJ	LOC02592	orf03885	LOC01469	SARI_01572	STM1409	-
		ssaK	LOC02537	orf03883	LOC01465	SARI_01570	STM1411	-
		ssaL	LOC02591	orf03882	LOC01471	SARI_01569	STM1412	-
		ssaM	LOC02590	-	LOC01473	-	STM1413	-
		ssaN	LOC02582; LOC04264	orf02130; orf03880	LOC01414; LOC05709	SARI_01567	STM1415	-
		ssaO	-	orf03879	LOC01470	SARI_01566	STM1416	-

		ssaP	LOC05051	orf03878	LOC04621	SARI_01565	STM1417	-
		ssaQ	LOC05045	orf03877	LOC01468	SARI_01564	STM1418	-
		ssaR	LOC02583; LOC04263	orf03876	LOC01466; LOC05705	SARI_01563	STM1419	-
		ssaS	LOC02734	-	LOC01459	SARI_01562	STM1420	-
		ssaT	LOC02580	orf03875	LOC01467	SARI_01561	STM1421	-
		ssaU	LOC05050	orf03874	LOC01463	SARI_01560	STM1422	-
		ssaV	LOC02588; LOC04271	orf02129; orf03881	LOC01464	SARI_01568	STM1414	-
		sscA	LOC02596	orf03895	LOC01476	SARI_01582	STM1399	-
		sscB	LOC02600	orf03891	LOC01479	SARI_01578	STM1403	-
		sseA	LOC02597	orf03897	LOC04633	SARI_01584	STM1397	-
		sseB	LOC02595	orf03896	LOC01484	SARI_01583	STM1398	-
		sseC	LOC05064	orf03894	LOC01478	SARI_01581	STM1400	-
		sseD	LOC05054	-	LOC01480	SARI_01580	STM1401	-
		sseE	LOC05055	orf03892	LOC01482	SARI_01579	STM1402	-
		ssrA	LOC05884	orf03901	LOC04631	SARI_01589	STM1392	-
		ssrB	-	orf03902	LOC01483	SARI_01590	STM1391	-
	TTSS effectors translocated via both systems	slrP	LOC05311	orf02900	-	SARI_02125	STM0800	-
		sspH1	-	-	-	-		-
	TTSS-1 translocated effectors	avrA	LOC05809	orf00879	-	-	STM2865	-
		sipA	LOC04350	orf00861	LOC05393	SARI_00091	STM2882	-
		sipB	LOC00924	orf00858	LOC03353	SARI_00088	STM2885	-
		sipC	LOC00921	orf00859	LOC05394	SARI_00089	STM2884	-
		sopA	-	-	-	-	STM2066	-
		sopB/sigD	LOC05351	orf04574	-	SARI_01910	STM1091	-

		sopD	LOC00971	orf00780	LOC01985	SARI_00017	STM2945	-
		sopE2	LOC04873	orf00414	LOC04293	SARI_01086	STM1855	-
		sopE	-	-	-	-	-	-
		sptP	-	orf00865	-	SARI_00095	STM2878	-
	TTSS-2 translocated effectors	gogB	-	-	-	SARI_00897	STM2584	-
		pipB2	-	-	LOC06113	SARI_00196	STM2780	-
		pipB	LOC05218	orf00093	-	SARI_01918	STM1088	-
		sifA	-	-	LOC05323	SARI_01766	STM1224	-
		sifB	-	-	LOC00121	SARI_01376	STM1602	-
		sopD2	-	-	-	SARI_01991	-	-
		spiC/ssaB	LOC02601	-	LOC01486	SARI_01588	STM1393	-
		sseF	LOC05058	orf03890	LOC04624	SARI_01577	STM1404	-
		sseG	LOC05059	orf03889	LOC04626	SARI_01576	STM1405	-
		sseI/srfH	-	-	-	SARI_02393	STM1051	-
		sseJ	LOC05220	orf00124	LOC04220	SARI_01350	STM1631	-
		sseK1	-	-	-	SARI_03503	STM4157	-
		sseK2	LOC05088	orf03961	LOC06047	-	STM2137	-
		sseL	-	-	LOC01358	SARI_00605	STM2287	-
		sspH2	LOC05343	orf04533	LOC05654	SARI_00212; SARI_00292; SARI_00895; SARI_01080	STM2241	-
	ACE T6SS(Escherichia)		-	-	LOC05823		-	-
			-	-	LOC04685		-	-
		aec15	-	-	LOC04689		-	-
		aec24	-	-	LOC05816		-	-
		aec25	-	-	LOC05818		-	-
		aec26	-	-	LOC05817		-	-

		aec27/clpV	-	-	LOC05815		-	-
		aec28	-	-	LOC05819		-	-
		aec29	-	-	LOC05820		-	-
		aec30	-	-	LOC06136		-	-
		aec31	-	-	LOC05824		-	-
		aec32	-	-	LOC06059		-	-
	LEE locus encoded TTSS(Escherichia)		-	-	LOC05686		-	-
			-	-	LOC05698		-	-
		cesD2	-	-	LOC05693		-	-
		cesD	-	-	LOC05706		-	-
		cesT	-	-	LOC05699		-	-
		escC	-	-	LOC05707		-	-
		escD	-	-	LOC06122		-	-
		escF	-	-	LOC05684		-	-
		escJ	-	-	LOC05712		-	-
		escU	-	-	LOC05703		-	-
		escV	-	-	LOC05708		-	-
		espA	-	-	LOC05695		-	-
		espD	-	-	LOC05689		-	-
		glrA	-	-	LOC06123		-	-
		glrR	-	-	LOC05677		-	-
		ler	-	-	LOC05680		-	-
		sepD	-	-	LOC05710		-	-
		sepL	-	-	LOC05696		-	-
		escS	LOC04260	-	-	-	-	-
	SCI-I T6SS(Escherichia)		LOC05696	orf03781	LOC04301	-	-	-
			LOC05691	orf03776	-	-	-	-
	T6SS-I(Klebsiella)		LOC04424	orf00672	-	-	-	-

	T6SS-III(Klebsiella)		LOC05697	orf03779	-	-	-	-
	TTSS (chromosomally encoded)(Yersinia)		LOC04279	orf02114	-	-	-	-
Serum resistance	Rck	rck	-	-	-	-	-	PSLT009
Spv locus	Spv	spvA	-	-	-	SARI_01480	-	PSLT040
		spvB	-	-	-	SARI_01481	-	PSLT039
		spvC	-	-	-	SARI_01482	-	PSLT038
		spvD	-	-	-	-	-	PSLT037
		spvR	-	-	-	SARI_01478	-	PSLT041
Toxin	Typhoid toxin	cdtB	LOC04878	orf00428	LOC04785	SARI_02386	-	-
		pltA	LOC04886	orf00432	LOC04782	SARI_02389	-	-
		pltB	LOC04881	-	-	SARI_02390	-	-
	Colicin-like Usp(Escherichia)	usp	-	-	LOC05997	-	-	-
Adherence	LPS O-antigen (P. aeruginosa)(Pseudomonas)		LOC04723; LOC04736; LOC04739; LOC04740	orf02785; orf02790; orf02794; orf02796	-	-	-	-
	Type IV pili(Yersinia)	pilQ	-	orf04356	-	-	-	-
		pilR	-	orf04357	-	-	-	-
		pilS	-	orf04358	-	-	-	-

		pilW	LOC04665; LOC05761	orf01110; orf02026	-	-	-	-
Glycosylation system	O-linked flagellar glycosylation(Campylobacter)	neuB2	LOC04730	orf02786	-	-	-	-
Immune evasion	Capsule(Acinetobacter)		LOC04726; LOC04727; LOC04732; LOC04737; LOC04744	orf02783; orf02784; orf02787; orf02798; orf02799	-	-	-	-
	LPS(Brucella)	wzt	LOC05144	orf04101	-	-	-	-
Iron acquision	Yersiniabactin(Klebsiella)		LOC04841	orf00319	-	-	-	-
Iron uptake	Acinetobactin(Acinetobacter)	basG	-	orf01667	-	-	-	-
	Iron-regulated element(Escherichia)	ireA	-	orf01637	-	-	-	-
	Iron/managanease transport(Escherichia)	sitA	-	orf04720	-	-	-	-
	Yersiniabactin siderophore(Escherichia)	irp2	LOC04830	orf00315	-	-	-	-
		ybtT	LOC04838	orf00318	-	-	-	-

	Yersiniabactin(Yersinia)	irp1	LOC04825	orf00316	-	-	-	-
		psn/fyuA	LOC04842	orf00320	-	-	-	-
		ybtA	LOC04831	orf00314	-	-	-	-
		ybtP	LOC04835	orf00313	-	-	-	-
		ybtQ	LOC04827	orf00312	-	-	-	-
		ybtS	LOC04833	orf00310	-	-	-	-
		ybtU	LOC04837	orf00317	-	-	-	-
		ybtX	LOC04834	orf00311	-	-	-	-
Non-LEE encoded TTSS effectors	NleC(Escherichia)	nleC	LOC05273	orf00211	-	-	-	-
Adherence	Intimin(Escherichia)	eae	-	-	LOC05692	-	-	-
Antiphagocytosis	Capsular polysaccharide(Vibrio)	wbjD/wecB	-	-	LOC05025	-	-	-
Autotransporter	EhaB	ehaB	-	-	LOC04396; LOC05796	-	-	-
Invasion	Invasion of brain endothelial cells (Ibes)(Escherichia)	ibeB	-	-	LOC04348	-	-	-

Appendix 08. Declaration of Copyright Ownership and/or Authorship.

Declaração

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Campinas, 04 de Setembro de 2020

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Appendix 09. Ethics Committee Approval.

UNICAMP - CAMPUS CAMPINAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização Fenotípica e Molecular de Amostras de Salmonella enterica subsp. enterica: Definição de clones de S. enterica 4,[5],12:i:-

Pesquisador: ALINE PAROLIN CALARGA

Área Temática:

Versão: 2

CAAE: 91276318.2.0000.5404

Instituição Proponente: Universidade Estadual de Campinas - UNICAMP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.919.668

Apresentação do Projeto:

1.1. O gênero SalmonellaO gênero Salmonella pertence à família Enterobacteriaceae que agrupa bacilos Gram-negativos, anaeróbios facultativos, fermentadores e geralmente flagelados (Crump et al., 2015; Mastroeni e Maskell, 2006). O esquema de White (1926) e Kauffmann (1978) divide o gênero Salmonella em tipos sorológicos (sorovariedades) definidos pela identificação de antígenos somáticos (O), flagelares (H) e capsulares (Vi). Os antígenos capsulares são característicos de alguns isolados e quando presentes definem uma sorovariedade. Já foram descritas 2541 sorovariedades de Salmonella (Agbaje et al., 2011).A nomenclatura e a taxonomia do gênero Salmonella modificaram-se desde a proposta que classificava cada sorovariedade como uma espécie (Kauffman, 1978; White, 1926). Com o uso de técnicas de biologia molecular associadas a testes bioquímicos, uma análise taxonômica mais apurada foi realizada (Agbaje et al., 2011). Todas as sorovariedades foram agrupadas em duas espécies: S. enterica e S. bongori. S. enterica subdivide-se em seis subespécies ou grupos (enterica – grupo I, salamae – grupo II, arizonae – grupo IIIa, diarizonae – grupo IIIb, houtenae – grupo V, indica – grupo VI) (Garai et al., 2012). De qualquer forma, o esquema de identificação baseado em sorovariedades ainda é muito utilizado e útil em estudos epidemiológicos. 1.2. Salmonelose S. enterica é um dos patógenos de origem alimentar mais prevalente. As fontes mais comuns de infecção são produtos contaminados derivados de aves domésticas, de carne suína ou bovina e mesmo plantas (Crump et al., 2015; Ritter and Tondo, 2014; Chen et al., 2013; Ruby et al., 2012).As sorovariedades de S. enterica

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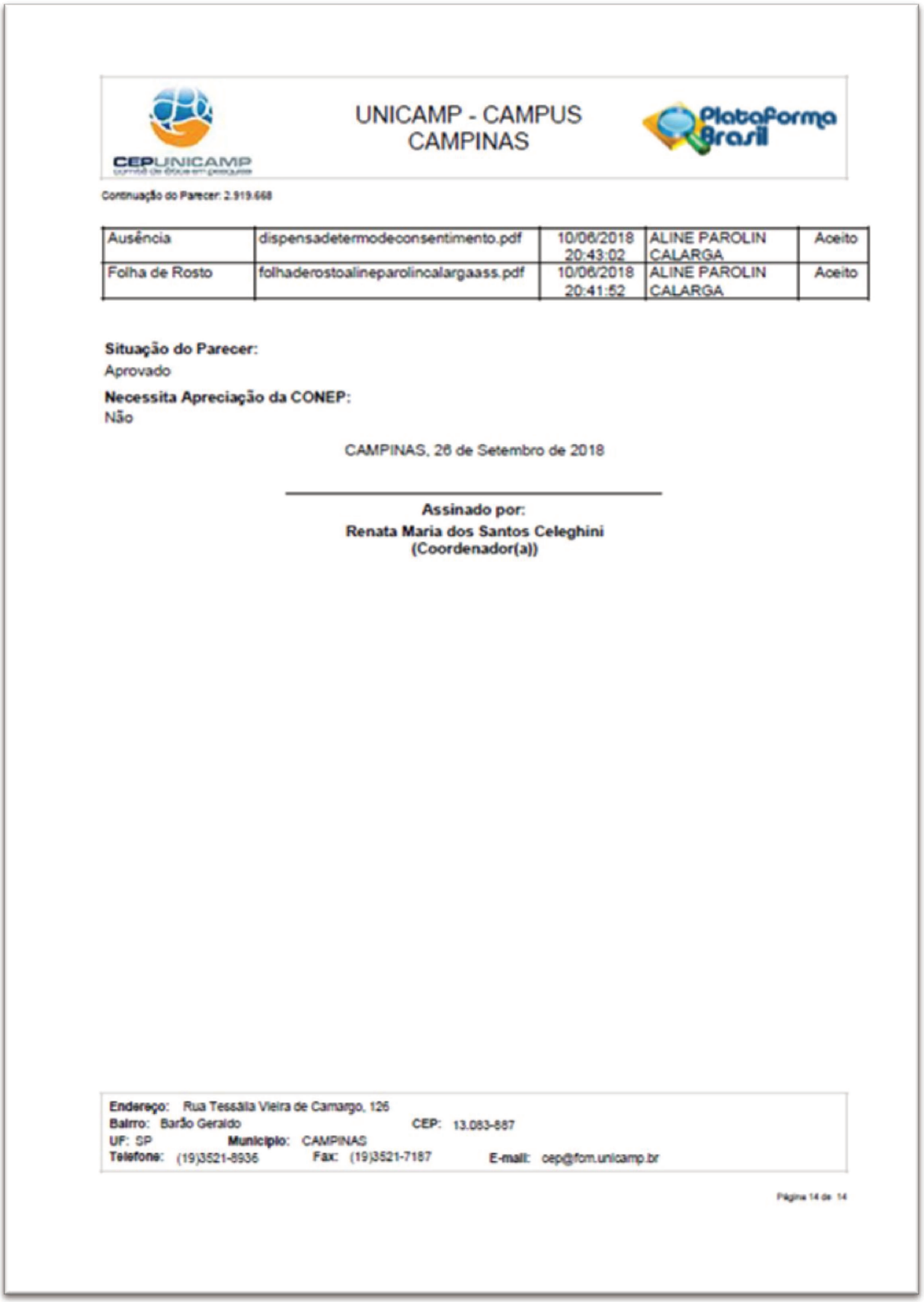

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Continuação do Parecer: 2.919.668

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CAMPINAS, 26 de Setembro de 2018

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador(a))

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