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REVIEW ARTICLE

Virulence Factors in *Salmonella* Typhimurium: The Sagacity of a Bacterium

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Abstract

Currently, *Salmonella enterica* Typhimurium (ST) is responsible for most cases of food poisoning in several countries. It is characterized as a non-specific zoonotic bacterium that can infect both humans and animals and although most of the infections caused by this microorganism cause only a self-limiting gastroenteritis, some ST strains have been shown to be invasive, crossing the intestinal wall and reaching the systemic circulation. This unusual pathogenicity ability is closely related to ST virulence factors. This review aims to portray the main virulence factors in *Salmonella* Typhimurium, in order to better understand the strategies that this pathogen uses to reach the systemic circulation and increase its infectivity in humans and animals. Thus, the most studied *Salmonella* pathogenicity islands in *Salmonella* Typhimurium were detailed as to the functions of their encoded virulence factors. In addition, available knowledge on virulence plasmid was also compiled, as well as the chromosome regions involved in the virulence of this bacterium.

Introduction

Salmonella spp. represents one of the principal causes of food poisoning in several countries in the last 100 years [72]. Approximately 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis, and 3 million deaths involving this bacterium are reported annually worldwide [83]. Due to its endemicity, high morbidity and, especially, difficulty in applying control and prevention measures, salmonellosis is characterized as an important zoonosis in a public health context [90].

The genus *Salmonella*, belonging to the *Enterobacteriaceae* family, includes two species, *S. enterica* and *S. bongori* [90]. The first comprises six subspecies designated by Roman numerals [25], and includes more than 2600 serotypes that differ from each other by their flagellar (H) and somatic (O) structures [17]. *S. enterica* subspecies I (*enterica*) is the most isolated subspecies in animals, and is found in 99% of human isolates. *S. bongori*, on the other hand, is often found in "cold-blooded" animals, such as reptiles, amphibians, and fish, and accounts for less than 1% of human isolates [83].

Diseases related to *Salmonella enterica* can be divided into three groups: Typhoid fever, caused by *S.* Typhi; enteric fever, caused by *S.* Paratyphi A, B, and C; and enterocolitis or salmonellosis, caused by the other serotypes [90]. According to Connor and Schwartz [19], because their symptoms are indistinguishable in a clinical analysis, both *S.* Typhi and *S.* Paratyphi are classified as causative agents for typhoid fever. These bacteria have human and some larger primates as their only reservoirs [33]. Symptoms are characterized by abdominal pain, headaches, fever, and diarrhea that may appear within a week of incubation [25]. In contrast, salmonellosis affects both human and animals and is the main cause of gastroenteritis in humans [1], resulting in 93.8 million cases, with 155,000 deaths each year [25].

Among these bacteria, the most frequently isolated serotypes are *S. enterica* Typhimurium (ST) and *S. enterica* Enteritidis (SE) [35]. In Brazil, ST was the most isolated serotype up to the mid-1990s, being surpassed after this by SE [1]. ST is a general pathogen, and can be isolated from different animals, as well as different foodstuffs. Thus, this serotype can be found in a variety of foods, and is mainly present in poultry [70, 103], swine

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[75, 95], and bovine meat [84]. However, some ST strains, in addition to causing localized gastroenteritis, are capable of causing systemic infections, such as the case of the multi-locus sequence type 313 (ST313) isolated in sub-Saharan Africa [61], and the most commonly found ST19 [12]. These bacteria multiply in the small intestine and extend beyond the intestinal wall, reaching the mesenteric lymph nodes and from there, the liver and spleen, where they settle and multiply [44].

It is believed that such an ability was acquired from horizontal gene transfer (transduction, conjugation, or transformation) over millions of years [33]. An example for this reasoning is the fact that ST and *S*. Typhi share about 90% of the genes present in their DNA [57]. This similarity allows ST to be used as a model for the study of typhoid fever in animals, since *S*. Typhi is human-specific [88]. The remaining 10% include virulence factors, defined as structures, products, and strategies that contribute to infection and that determine the degree of pathogenicity of this serotype [57]. In ST, most of the genes that encode such factors are located in the so-called *Salmonella* Pathogenicity Islands (SPI) [76]. Although less relevant, they may also be present in other parts of the chromosome, such as the fimbriae and flagella. The genes encoding these characteristics can be found in virulence plasmids (pSLT), more precisely in the *spv* operon [26].

In this context, this review aims to portray the main virulence factors in *Salmonella* Typhimurium in order to better understand what strategies this pathogen uses to reach the systemic circulation and increase its infectivity in humans and animals (Table 1).

Overview of the Infection Process

Infection caused by ST occurs mainly through the ingestion of contaminated animal or water food [11, 76]. Upon reaching the stomach, this microorganism must cope with the acidity of the medium, so its acid tolerance response (ATR) is activated, which maintains the intracellular pH higher than the extracellular pH, allowing the bacterium to survive while in this environment [26]. Subsequently, ST crosses the mucus layer present in the intestinal wall and adheres to the epithelium [7, 48], where the infection will occur. The interaction of ST with the epithelium results in the appearance of the clinical diagnosis, characterized by diarrhea, culminating in the loss of electrolytes and inducing local inflammation of the intestine [46].

ST adhesion to the epithelium is achieved by host–receptor interactions with many of the adhesion factors present on the cell surface of this microorganism [76]. After this stage, effector proteins are released into the enterocyte cytoplasm, causing changes in the cytoskeleton of the intestinal epithelium [76]. These modifications lead to the formation of membrane extensions, known as ruffles, similar to those formed by phagocytic cells [25]. The ruffles encompass ST and launch it into the cell [11]. With the engulfment of the bacterium by enterocytes, intracellular phagosomal compartments, termed *Salmonella*-Containing Vacuoles (SCV), are formed [24, 31, 44, 46, 50, 67] (Fig. 1). This compartment is the only place in these host cells where ST the can survive and multiply [48]. As a consequence of this invasion, transcription activators are generated, leading to the production of proinflammatory cytokines, such as interleukin (IL)-8, which ultimately induce the inflammatory response [46]. With this induction, polymorphonuclear leucocytes (PMN)

Table 1 Components of T3SS-1 and T3SS-2, substructures of its components (if any), and structural proteins that comprise those components

Components	Substructures	Protein components T3SS-1	Protein components T3SS-2	References
Needle complex	Needle structure	PrgI	SsaG	[23, 60]
		PrgJ	SsaI	
Needle complex	Inner membrane rings	PrgK	SsaJ	[23, 60]
		PrgH	SsaD	
	Outer membrane rings	InvG	SsaC	
Export apparatus		SpaO	SsaQ	[23, 60]
		SpaP	SsaR	
		SpaQ	SsaS	
		SpaR	SsaT	
		SpaS	SsaU	
		InvA	SsaV	
		InvC	SsaN	
		OrgA	–	
Translocon		SipBCD	SseCDB	[23, 60]

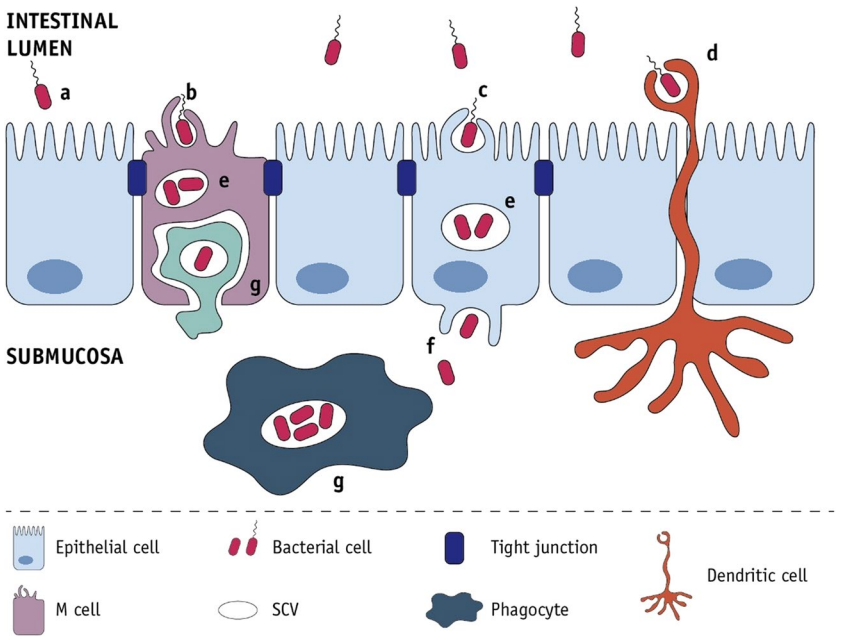

Fig. 1 Pathogenesis of *Salmonella* Typhimurium. **a** *Salmonella* adheres to the intestinal epithelial and M cells using many of adhesions factors present on its cell surface. **b, c** Effector proteins are released into enterocyte causing changes on its cytoskeleton and forming structures in its surface known as ruffles. **d** Alternatively, the bacterial cells can be directly taken by dendritic cell from the submucosa. **e** Once inside cytoplasm, *Salmonella* cells are located into SCV (*Salmonella* Containing Vacuoles), where it multiplies. **f** The SCV transcytose to the basolateral membrane and release to the submucosa. **g** Bacteria is internalized within phagocytes and then located again into SCV; this figure was based on the one illustrated in the article [89]

migrate into the intestine and induce the production of antimicrobial substances, where apparently ST is less affected [52]. This generates a microenvironment in which ST has a growth advantage over the resident microbiota, which is also affected by the substances released by PMNs [7].

Jones et al. [56] noted that salmonellae adhere to and preferentially invade M cells, which are modified cells of the intestinal wall of the ileum present in Peyer's plaques. M cells are intimately associated with macrophages, which reside in this tissue [53]. When the epithelial barrier is ruptured, ST can invade macrophages and dendritic cells and, from these infected cells, gain systemic circulation [52]. During all stages of this infection, ST uses specific virulence factors to conduct a more efficient invasion (Table 2). In this context, we will now report these factors and their roles in infection caused by *Salmonella* Typhimurium.

Strategies and Virulence Factors

Type III Secretion System (T3SS)

Salmonella spp. possesses a molecular apparatus called the Type III Secretion System (T3SS) [17, 44], which is responsible for the ability of these microorganisms to inject effector proteins into the cytosol of host cells and modulate host cells signaling cascades for the benefit of bacteria [17, 57]. These effectors, once within the cell, can alter cellular functions such as cytoskeleton structure, membrane transport, signal transduction, and cytokine expression [48]. These changes allow for the invasion and permanence of the bacterium in the infected cell [17]. T3SS comprises several components (Table 1), including more than twenty proteins [48], some of them are homologous to those involved in flagellar assembly [77], suggesting a evolutionary relation [66].

Salmonella enterica abstract two distinct T3SSs (T3SS-1 and T3SS-2), which are encoded by SPI-1 and SPI-2, respectively, located in different *Salmonella* DNA clusters [46]. They have shown to function at different times during the infection [46]. Thereby, T3SS-1 is active with the contact of the bacterium with the host cell membrane and translocates effector proteins into its cytoplasm, while T3SS-2 is active within the phagosome and translocates effectors into the vacuolar space [48]. This ability could be very important for systemic increase of ST in its host, once T3SS-2 secreted genes are required for bacterial growth in macrophages [16, 51, 82]. Most of the effectors translocated by T3SS-1 are encoded on SPI-1, but, as we will show in this article, some of them can be encoded on SPI- 5 [46], as well as some also can be encoded in other parts of ST DNA [77]. Just like T3SS-1, T3SS-2 translocates effectors which are encoded in other parts of ST DNA [99], despites most of the effectors

Table 2 Main virulence factors, DNA location, and main functions

Virulence factor	Location	Function	Reference
SipA	SPI-1[a]	Cytoskeleton rearrangement	[104]
		Chemotaxis	[76]
SipB	SPI-1	Translocation of effector proteins	[30]
		Macrophage apoptosis impairment	[18]
SipC	SPI-1	Chemotaxis	[76]
		Cytoskeleton rearrangement	
SptP	SPI-1	Suppression of innate immunity	[14]
trr genes	SPI-2	Production of tetrathionate reductase	[26]
SpiC	SPI-2	Translocation of effector proteins	[28]
		Survival within SCV[c]	[98]
SseB	SPI-2	Formation of macromolecular structures which serves as a translocon	[70]
SseC	SPI-2	Formation of macromolecular structures which serves as a translocon	[70]
SseD	SPI-2	Formation of macromolecular structures which serves as a translocon	[70]
SseF	SPI-2	SCV perinuclear migration	[68, 69]
		Microtubule aggregation	
		SIF formation[b]	
SseG	SPI-2	SCV perinuclear migration	[68, 69]
		Microtubule aggregation	
		SIF formation	
MisL	SPI-3	Long-term persistence	[24]
MgtCB	SPI-3	Survival within macrophages	[5]
MarT	SPI-3	Activation of MisL expression	[85]
SiiE	SPI-4	Adhesion to the epithelium	[34]
SopB	SPI-5	Prevents apoptosis of epithelial cells	[64]
SigE	SPI-5	Chaperone	[21]
SpvR	pSLT	Regulation of *spv* genes	[38]
SpvB	pSLT	Prevents actin polymerization	[38]
SpvC	pSLT	Inhibits MAP kinase and immune signaling	[91]
Type I Fimbrae	Chromosome	Adhesion to the epithelium	[3]
SifA	Chromosome	SIF formation	[4]
		SCV maintenance	
SseJ	Chromosome	SIF formation	[69]
SopE	Chromosome	Induce membrane ruffling in cell cultures	[47]
SopE2	Chromosome	Induce membrane ruffling in cell cultures	[47]

[a]SPI *Salmonella* pathogenicity island

[b]SIF *Salmonella*-induced filaments

[c]SCV *Salmonella*-containing vacuoles

translocated for this system is encoded on SPI-2 [51]. Although T3SS-1 and T3SS-2 have similar structures and perform the same function (translocate effectors proteins), these systems are not identical themselves, since both have different structural proteins and, as said, secrete different effector proteins [99]. Their structural proteins, however, share some homology (Table 1), which makes their mechanisms not seem significantly different [71]. It is believed, though, that they are not independent one of another, once mutations in T3SS-2 causes a significant reduction in the expression of some genes of T3SS-1, which harms the ability of the bacterium to invade epithelial cells [22]. In view of its homology, we prefer to use T3SS-1 of ST as an archetype to prevent confusion and since T3SS-1 of ST is, till now, the most well-characterized system [20, 59, 60, 66, 67]. The corresponding components of T3SS-2 can be found in Table 1.

Kubori et al. [61], after observing the structure of T3SS-1 apparatus under optical microscopy, has found a supramolecular structure of ST T3SS-1 apparatus called the Needle Complex (NC) [48]. The NC is, by definition, a hollow structure composed by two pairs of rings that are anchored

to the inner and the outer membranes of the bacterium, and a needle-like structure that extends beyond the surface of the bacterium [59] (Fig. 2). A NC is composed of at least five proteins components: PrgI, J, K, H, and InvG [60], each one of them is required for the type III secretion and the invasion of epithelial cells [59]. PrgI was demonstrated to be component of the needle portion of the NC, since ST *prgI* mutant strain did not present needle substructures, although it did exhibit apparently normal bases [67]. Beyond that, the same study has showed that PrgI is essential for ST entry into host cells, once *prgI* mutant was completely defective in its ability to invade cultured intestinal cells [67]. PrgJ is required for PrgI secretion as well as the formation of the needle structure, and it is also the minor component of the needle itself [60]. PrgH and PrgK, on the other hand, interact with each other to form the inner membrane rings of the NC [59]. Lastly, InvG, which is a member of the secretin family of protein [67], forms the outer membrane rings of the NC, which are believed to permit the passage of exported

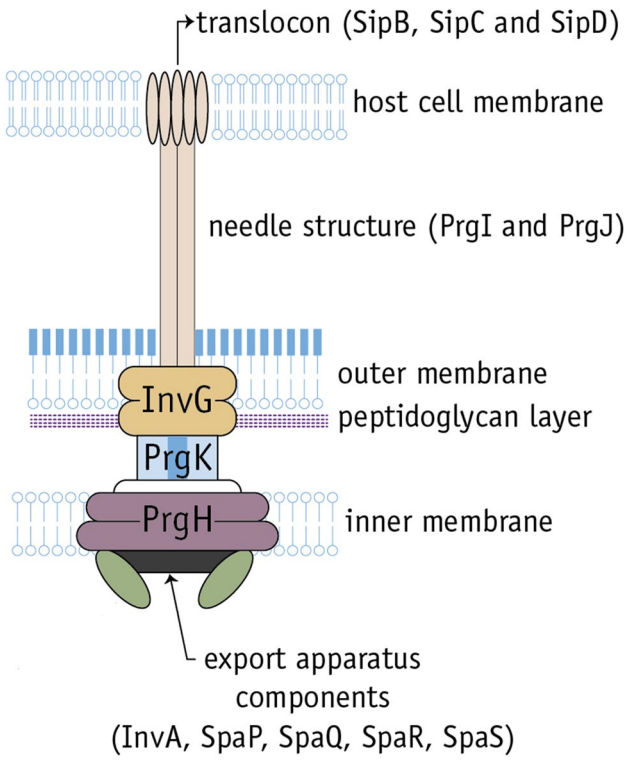

Fig. 2 T3SS-1 structure and its components. A Translocon, which forms a translocation pore in the host cell membrane and translocates effector proteins into the host cytoplasm, formed by SipB, C, and D proteins; a needle structure, which extends beyond the surface of the bacterium, formed by PrgI and PrgJ proteins; an outer membrane rings, formed by InvG, located in outer membrane; and an inner membrane rings, formed by PrgK and PrgH, located in the inner membrane. In addition, an Export Apparatus, housed within the inner membrane rings, formed by the proteins summarized in Table 1; this figure was based on the one illustrated in the article [31]

substrates across the outer membrane [60]. Housed within the inner membrane rings of the NC base, there is a Type III Export Apparatus which is required to assembly of the NC and the secretion of effector proteins [94]. This apparatus is believed to be composed by eight proteins (SpaO, P, Q, R, S, InvA, C, and OrgA), which are conserved among all T3SS [55]. A few sets of other proteins (as SipBCD, explained in the following section) comprise a Translocon, which is believed to be involved in the translocation of effector proteins into the host cytoplasm by forming a translocation pore in the host cellular membrane [48, 60].

Salmonella Pathogenicity Islands

Salmonella pathogenicity islands (SPIs) are chromosomal regions that carry virulence genes that act as a compact and distinct genetic unit known as an operon [77]. These regions display a different composition from the rest of the chromosome, with the characteristic presence of a greater amount of G+C (guanine and cytosine) when compared with the other parts of the DNA [79]. To date, five SPIs have been described in ST, with SPI-1 and SPI-2 being the most recognized and studied [2, 17, 25, 44, 77]. Although the other three SPIs have not been studied as closely as the first two [26], studies have reported the involvement of these islands in *Salmonella* invasion of and survival within host cells [11].

SPI-1 is the most well-characterized island among the five ST SPIs [26, 33, 77]. Genes encoded by this region are said to be essential in the invasion stage through the intestinal epithelium [77], since salmonellae SPI-1 mutants have shown an attenuation for systemic invasion when inoculated orally in rats. However, this does not occur when inoculation is performed by the parenteral route [31]. This suggests that these genes are closely related to the host's internalization of the bacterium and crucial for ST to penetrate the intestinal wall and consolidate the invasion [31]. SipABCD and SptP are the major effector proteins encoded by these genes, released into the host cell through T3SS-1.

SipA is said to be one of the first proteins responsible for the induction of cytoskeletal rearrangement of epithelial cells, which facilitates bacteria entry into the host cell [104]. This has been confirmed by in vitro studies with ST *sipA* mutants, in which it was demonstrated that such a microorganism was less effective in inducing actin-cytoskeletal reorganization in HeLa cells, in the same way that they were prevented from being internalized by these cells [104]. As with SipA, SipC is also responsible for inducing actin-cytoskeletal rearrangements [76] and, alongside SipB, is involved in T3SS-1 translocation of effector molecules (e.g., SptP) into the host cell [18]. Studies conducted with *sipB, sipC sipD* mutants have shown the inability of these strains to stimulate any response in the T3SS-1-dependent cell [18]. In addition, SipA and SipC also activate a signal transduction

cascade that leads to PMN migration [76], creating a microenvironment favorable for ST growth [7]. The role of SipB is still not fully elucidated [76]; however, Hersh et al. [53] describe its ability to induce macrophage apoptosis from the activation of caspase-1, and stated that *Salmonella*-induced macrophage cytotoxicity is SipB-dependent.

Another protein encoded by genes located in SPI-1 is SptP. This effector is a phosphotyrosine phosphatase which, when injected into epithelial cells, alters the actin cytoskeleton [30]. Nevertheless, as demonstrated by Kaniga et al. [58], SptP is not required for host cell invasion, since *sptP* ST mutant strains enter cultured epithelial cells and macrophages with the same efficiency as wild-type strains. Likewise, *sptP* mutants did not display the same efficacy in colonizing the liver of infected mice compared to wild-type strains [58]. More recently, Choi et al. [14] reported the role of SptP in suppressing the innate immunity of the host, demonstrating that it is able to prevent mast cell degranulation, which may be an important mechanism of ST virulence.

SPI-2 is a pathogenicity island that shows great importance in the maintenance of its permanence in the host cell. This chromosome sequence contains about 40 genes [77], grouped into four operons: *ssa* (type 3 secretion system), *ssr* (secretion system regulation), *ssc* (chaperone secretion), and *sse* (effector coding) [51].

SPI-2 is divided into two segments, one large and one small [26]. The latter is characterized by the presence of *trr* genes, which are involved in the production of tetrathionate reductase, responsible for *Salmonella*-like tetrathionate respiration [50] and seven open reading frames (ORFs) [26], which, to date, have not demonstrated a significant role in ST virulence [26]. On the other hand, the major follow-up displays some importance in the ability of ST to survive and multiply within SCV in host cells, either epithelial or macrophages [82]. Fields et al. [27] reported that salmonellae deficient in macrophage replication display attenuated virulence, corroborating the idea that this capacity is essential for *Salmonella* pathogenesis. Similarly, Cirillo et al. [16] have shown that SPI-2 mutants can colonize Peyer's plaques, but are unable to colonize the liver, spleen, or mesenteric lymph nodes, which require invasion into macrophages. This ability is given, mainly, by effector proteins SpiC, SseF, and SseG that are translocated by T3SS-2.

Uchiya et al. [98] reported the first protein encoded by SPI-2 and translocated via T3SS-2 to the macrophage cytoplasm, SpiC. In their study, SpiC was classified as the protein responsible for the inhibition of phagolysosome formation (junction of lysosome-containing vacuoles with the SCV). This mechanism enabled ST to be able to survive for more than 24 h within macrophages and dendritic cells [45]. Uchiya et al. [98] also demonstrated that *spiC* is of great importance for ST virulence, since its deletion resulted in a considerable attenuation of this characteristic. According to Freeman et al. [28], SpiC is still required for the translocation of effector proteins inside host cells, such as SseB and SseC.

Effector proteins SseB, SseC, and SseD have been shown to be secreted by T3SS-2 when ST is cultured in vitro under SPI-2-inducing conditions [63]. These same proteins are found on the cell surface of the bacterium and are necessary for translocation of other effector proteins to the infected cell [80]. They are believed to form a macromolecular structure in the membrane that serves as a translocon [80]. In addition, SseC and SseD have also been shown to be essential for virulence, since *sseC* and *sseD* mutants display a decrease in this characteristic [63].

Hensel et al. [51], when analyzing *sseF* and *sseG* mutants, noted that these genes play a role in systemic pathogenesis and intracellular proliferation. In addition, Kuhle and Hensel [68] demonstrated that *sseF* and *sseG* may be related to the induction of *Salmonella*-induced filaments (SIF), since their exclusion leads to a considerable decrease in the formation of these structures when compared to wild-type strains. SIF are structures that arise when, a few hours after internalization, SCV begin to elongate into tubular filaments [8]. Their formation involves the fusion of late endocytic compartments [10] and, although this is clear, the mechanisms that mediate this process are still unknown [8]. Studies, however, suggest that microtubules serve as a support for such a formation [69]. Finally, Kuhle et al. [69] demonstrated that SseF and SseG are co-localized with microtubules, present in endosomal compartments that aggregate to SIF along these same structures, further corroborating the hypothesis postulated by Kuhle and Hensel [68].

As mentioned previously, the other SPIs has not been described in as much detail as the first two, although some information is available. SPI-3, for example, contains ten ORFs organized into six transcriptional units [77]. The proteins encoded by these genes, however, play roles with no obvious relation to each other [6]. *mgtCB*, for example, encodes proteins that are required for survival within macrophages [5], thus contributing to its virulence. On the other hand, MisL, a protein encoded in SPI-3, contributes to intestinal colonization and is required for long-term intestinal persistence and involved in host–pathogen interactions during infection [24]. In addition, MarT has demonstrated regulatory functions [26], in addition to activating MisL expression [97].

SPI-4 contains six ORFs arranged in a single operon, called *siiABCDEF* [26]. Morgan et al. [78] demonstrated that, although not necessary for systemic infection, SPI-4 is required for intestinal colonization. Taking this into consideration, Gerlach et al. [34] analyzed the role of SPI-4-encoded proteins in the interaction with epithelial cells and demonstrated that this island plays an important role in adhesion to the intestinal epithelium. By infecting epithelial

cell lines (MDCK) with wild-type *Salmonella* and SPI-4-deficient strains, Gerlach et al. [34] observed that a considerable percentage (4.24%) of the wild-type *Salmonella* adhered to the epithelium, whereas only 0.28% of SPI-4-deficient *Salmonella* displayed this characteristic. Regarding the SiiE protein encoded by the *sii* operon, some non-fimbrial adhesin properties are displayed, evidenced by decreases in adhesion when the *siiE* gene is deleted [34]. In order to discover the individual role of each gene encoded by the *sii* operon, Kiss et al. [62] tested the virulence of mutants carrying non-polar deletions in individual *sii* genes with wild-type *Salmonella* strains. The results demonstrated that, even at different levels, wild-type strains were more virulent than the six *sii* mutants, suggesting that they all display some significance in *Salmonella* virulence. However, further studies should be conducted to find out the exact role of these genes in ST pathogenicity.

SPI-5 is composed of five genes (*pipD, sigD*/*sopB, sigE*/*pipC, pipB*, and *pipA*) [64], which may be related to the role of SPI-5 in enteropathogens [77]. Among the five coded proteins, SopB (SigD in ST) is the best described and known to be translocated by T3SS-1 into the host cell cytoplasm [46]. Galyov et al. [32] demonstrated that, although *sopB* deletion did not affect pathogen invasiveness, SopB plays an important role in the induction of fluid secretion by enterocytes, as well as in PMN induction in the intestine. In addition, SopB also presents inositol phosphate phosphatase activity, which is directly related to the induction of diarrhea [81]. Knodler et al. [65], on the other hand, demonstrated that SopB is able to prevent apoptosis of cultured epithelial cells from the activation of Akt, an important apoptosis regulator in these cells, in a mechanism used by the host to protect itself from a pathogen. SigE was characterized by Hong and Miller [54] as a possible chaperone that acts on the stability of SopB secretion. This hypothesis was later confirmed by Darwin et al. [21]. Finally, mutational studies were performed by Wood et al. [101] in order to discover the role of the *pipA, pipB*, and *pipD* genes. None of the mutants in these genes had their growth rate or ability to invade HeLa cells affected. However, both inflammatory responses and the rate of fluid secretion were reduced in *pip* mutants compared to wild-type strains. This suggests that these genes are directly linked to the *Salmonella* enteropathogenicity, although they do not have an effect on the development of systemic invasion [101].

Virulence Plasmids

Several *Salmonella enterica* carry virulence plasmids, including *S*. Enteritidis, *S*. Dublin, *S*. Choleraesuis, and *S*. Typhimurium [39–41]. Such plasmids are differentiated among serotypes according to their size [15]. In ST, the virulence plasmid contains 95 kb size [15] and is named pSLT [92, 96]. It is believed that this structure was acquired horizontally, since it is located in a region adjacent to the insertion element and contains 46% less G+C than other parts of the chromosome [77]. pSLT is equipped with a highly conserved 8-kb sequence, with five ORFs, named the *spv* operon (*Salmonella* plasmid of virulence) [40]. This small part of the plasmid, however small, can restore virulence in plasmid-cured salmonellae in a mouse model [41]. This information led several research groups to further study the role of *spv* genes in virulence, since strains lacking the *spv* operon proved to be avirulent [39]. Gulig and Doyle [42], for example, observed that *spv* genes were directly related to the ability of ST to increase its multiplication rate within the host during infection. Later, Guilloteau et al. [36] analyzed the interactions of many wild-type and plasmid-cured *Salmonella* serotypes in vitro murine and bovine macrophages and in vivo mice after infection. The presence of virulence plasmids increased the lytic activity in macrophages infected by ST, *S*. Dublin, and *S*. Choleraesuis, besides inducing inflammatory responses in the peritoneum. Gulig et al. [43], on the other hand, examined the relationship between *spv* genes and the rate of ST growth in different cell populations, including lymphocytes and neutrophils in addition to macrophages. Genetic markers were used to measure the number of cell divisions in cells infected with ST strains with or without the virulence plasmid. It was noted that only the macrophages showed relevance for ST multiplication [43], since, with the quantitative decrease of these cells, the two treatments (with and without the plasmid) showed the same results for virulence [43]. Recently, Wu et al. [102], based on analyses in zebrafish larvae, demonstrated the ability of wild-type strains of ST to inhibit the autophagic activity of macrophages and neutrophils, in addition to repressing the function of macrophages and neutrophils when compared to ST *spv* mutants. Thus, they demonstrated that the *spv* genes are also involved in the suppression of innate immune response of the host [102]. All these studies suggest the important role that the *spv* operon plays in ST virulence, although the mechanisms it applies are not yet fully understood [102].

However, some mechanisms of effector proteins encoded by *spv* operon have been elucidated. As mentioned previously, *spv* operon consists of five genes. The first one is *spvR*, which is also the first gene to be transcribed [41]. The effector protein it encodes, SpvR, has as main function regulating the expression of the other four genes [40]. This protein displays homology with MetR, a LysR member of positive regulatory proteins [37]. SpvB is a cytotoxic protein [26], which has the function of avoiding, above all, actin polymerization [38], being thus related to the intracellular phase of infection. Additionally, Li et al. [74] demonstrated the role of *spvB* in inhibiting autophagy in a zebrafish infection model. This mechanism results in an increase of *Salmonella*

virulence, since autophagy is a natural defense mechanism of organisms infected by this pathogen [74]. SpvB was also shown to be indispensable for virulence, since *spvB* mutants are avirulent in mice [86]. SpvC is an anti-inflammatory effector which plays an important role in the host's proinflammatory response [26], since it inhibits MAP kinases and, consequently, immune signaling [91]. Until recently, SpvD still did not have its role defined; however, recently Rolhion et al. [85] demonstrated the importance of this protein in the suppression of the immune response, interfering with the transport of nuclear machinery mediated by NF-κβ. Finally, SpvA still has no elucidated role in ST virulence, requiring further studies [86].

Fimbriae and Flagella

Fimbriae are structures found on the cell surface of some bacteria, which have been shown to play an important role in the formation of biofilms, colonization, and the initial attack of the bacterium on the host [57]. With the sequencing of the ST genome, 13 operons were discovered [*agf* (*csg*), *fim, lpf, pef, bcf, stb, stc, std, stf, sth, sti, saf*, and *stj*] with homology to fimbrial biosynthesis genes [26, 76]. Since then, several studies have been carried out to determine their action on the virulence of this pathogen. Bäumler et al. [3] used a genetic approach to investigate the role of three fimbrial operons (*fim, lpf*, and *pef*) in ST adhesion to different epithelial cell lines (HEp-2 and HeLa). These operons encode type I fimbriae, long polar fimbriae, and plasmid-encoded fimbriae, respectively [3]. The results showed a significant decrease in adhesion to HEp-2 cells only for *lpf* ST mutants, whereas the end deletion significantly decreased ST adhesion to HeLa. These results suggest that the bacterial repertoire of fimbrial adhesins determines which type of epithelial cell the bacterium will adhere to during ST intestine infection [3]. Weening et al. [100] tested the role of colonization of ten fimbrial operons (*fim, pef, lbf, bcf, stb, stc, std, stf, sth,* and *agf*) of the bacteria in the intestine. Genetically resistant mice (e.g., CBA) were submitted to fimbrial operon mutation strains and the results demonstrated that, from fimbrial operated operons, only *bcf, lpf, stb, stc, std,* and *sth* contribute to the ability of ST to be shed with the feces and to colonize the cecum after 30 days of infection [100]. However, these same data demonstrated that persistence in the intestine is complex and involves the expression of more than 32 ST genes [100]. Although we know the importance of these operons in virulence, the exact mechanisms remain to be elucidated.

The flagella is a long helical filament coupled to rotating motors embedded within the outer membrane and cell wall, which enables ST and the other bacteria that display this feature to mobilize through the epithelial barrier after ingestion [57]. It is characterized as a strong inflammation inducer, mainly from the induction of Interleukin (IL)-8 and activation of NF-κβ [17], a protein complex that plays the role of a transcription factor and is involved in the immune response to infection [73]. This induction is due to its chemotactic potential, which is also one of the main flagella characteristics [13, 26].

Factors Present in Other Parts of the Chromosome

Some effector proteins are encoded outside the SPIs and translocated to the host cell by T3SS. SopE (located in a cryptic prophage) and SopE2, for example, although not encoded in SPI-1, are translocated by T3SS-1 [77]. These, together with the Sip proteins, play an important role in ST invasion. Studies have demonstrated the ability of SopE to induce membrane ruffling of cell cultures and that this induction occurs from the GDP/GTP nucleotide exchange in the Rho family, the G protein family responsible for the intracellular dynamics of actin [49]. Because they are about 70% identical [47], SopE and SopE2 have similar roles in the invasion [46]. However, Friebel et al. [29] demonstrated that SopE and SopE2 activate different sets of RhoGTPases in the host cell, suggesting that this small difference is responsible for the existence of such similar proteins in the same bacterium.

Other important effector proteins for ST virulence are SifA and SseJ. These effectors are translocated into the host cell cytoplasm by T3SS-2 [99]. They seem to have some relation to each other [87] and their roles in ST virulence are similar. Studies in *sifA* [4, 9] and *sseJ* [87] mutants demonstrated an attenuation in their replication in macrophages when compared with wild-type strains. In addition, a few hours after internalization, *sifA* mutants appeared to display vacuolar membrane loss, with these organelles being found loose in the host cell cytosol [4]. This demonstrates the importance of this protein in both SCV maintenance and intracellular replication. Another study reported that SifA is also required for the formation of SIF [93] and, later, Kuhle et al. [69] reached the same conclusion for SseJ, demonstrating that SseJ, like SseG and SseF, is located along with the microtubules in host cells, which, as mentioned previously, serve as support for the formation of SIF.

Conclusions

Salmonella Typhimurium is an intracellular pathogen, and its ability to invade host cells and disseminate in the body is closely related to its virulence genes. Further study of such genes may allow for therapeutic measures against this microorganism to be developed, since the general knowledge of the mechanisms used by ST in the invasion puts us one step ahead in the fight against it. In light of what has been reported in this review, it is notable that several advances have been achieved

in the research on the main factors of ST virulence. Among these, it is perceived that SPIs play a crucial role in ST pathogenicity, being, therefore, the most studied. The main studies about the virulence of this pathogen are in the SPI-1 and SPI-2. The discovery that both encode T3SSs was of great value, since these apparatuses have the ability to inject not only effector proteins encoded in SPI-1 and 2, but also those encoded in other parts of the chromosome into the cytoplasm of the host cell. This fact further heightens the importance of these two islands in maintaining virulence, since their absence eliminates the ability of ST to inject T3SS-dependent effector proteins. Despite this, much evidence also points out the role of the other three SPIs, SPI-3, 4, and 5 on ST virulence. However, much of the knowledge about these islands is still based on empirical evidence, due to the absence of theoretical-scientific basis, which can only be acquired with more research in the area. An example of such structures are the SPI-4 *sii* genes and Pip proteins in SPI-5, in which the exact mechanisms behind the fact that their causes a certain attenuation in ST virulence are not yet fully elucidated.

On the other hand, it has been shown that pSLT is required for ST virulence. However, some mechanisms of certain proteins produced in the *spv* operon, such as SpvA, as well as their role in virulence, have not yet been fully elucidated, requiring more comprehensive studies. Likewise, 13 operons encoding fimbriae are also involved in ST virulence, contributing mainly to the adhesion of this bacterium to the host cell. However, the exact mechanisms used by each gene comprising this operon have not yet been discovered. Thus, it can be concluded that, although many advances have been made, there are still many challenges to be overcome regarding ST virulence factors.

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Compliance with Ethical Standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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