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Review

From *Eberthella typhi* to *Salmonella* Typhi: The Fascinating Journey of the Virulence and Pathogenicity of *Salmonella* Typhi

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Cite This: *ACS Omega* 2023, 8, 25674−25697

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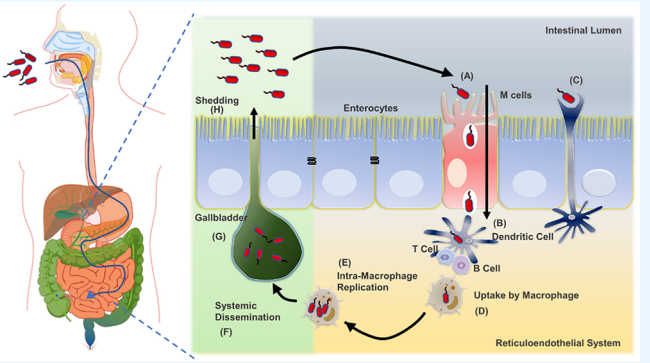
ABSTRACT: *Salmonella* Typhi (*S.* Typhi), the invasive typhoidal serovar of *Salmonella enterica* that causes typhoid fever in humans, is a severe threat to global health. It is one of the major causes of high morbidity and mortality in developing countries. According to recent WHO estimates, approximately 11−21 million typhoid fever illnesses occur annually worldwide, accounting for 0.12−0.16 million deaths. *Salmonella* infection can spread to healthy individuals by the consumption of contaminated food and water. Typhoid fever in humans sometimes is accompanied by several other critical extraintestinal complications related to the central nervous system, cardiovascular system, pulmonary system, and hepatobiliary system. *Salmonella* Pathogenicity Island-1 and *Salmonella* Pathogenicity Island-2 are the two genomic segments containing genes encoding virulent factors that regulate its invasion and systemic pathogenesis. This Review aims to shed light on a comparative analysis of the virulence and pathogenesis of the typhoidal and nontyphoidal serovars of *S. enterica.*



INTRODUCTION

Salmonella enterica, a Gram-negative food-borne pathogen causing enteric fever and gastroenteritis, is one of the significant reasons for thousands of mortalities worldwide.[1−3] *Salmonella* is divided into two species: *Salmonella bongori* and *Salmonella enterica*.[4] The species *enterica* is further categorized into six subspecies: *enterica*, *salamae*, *diarizonae*, *indica*, *arizonae*, and *houtenae*, with about 2700 serovars based on surface antigens. Almost 1500 serovars of *S. enterica,* which includes Typhimurium and Enteritidis, can cause infection in humans and animals after being consumed with contaminated food and water.[5] On the contrary, *S. bongori* can only infect cold-blooded animals. Among all the serovars, the *S. enterica* serovar Typhi (*S.* Typhi) infection is strictly restricted to humans (known as typhoid fever). The host-restriction of *S.* Typhi is attributable to a 5% reduction of its genome due to the pseudogenization of 200−300 genes.[6] Other typhoidal serovars, such as *S.* Paratyphi A, B, and C, are also associated with systemic infection in humans, known as paratyphoid fever.[7]

The nontyphoidal serovar (NTS) *Salmonella* Typhimurium (*S.* Typhimurium) can infect a wide range of hosts, including humans, plants, and animals.[8,9] Other serovars of *S. enterica*, such as Enteritidis, Gallinarum, and Pullorum, are responsible for the outbreaks in poultry industries.[10,11] Additionally, certain strains of *Salmonella,* such as sequence types ST313, ST34 (for Typhimurium), and ST11 (for Enteritidis), are associated with invasive bloodstream infection in humans.[12] It has been reported that the multidrug-resistant *S.* Typhimurium ST313 causes bloodstream infection in adults and children with 20−25% fatality in sub-Saharan Africa.[13,14] *Salmonella* infection in humans can also cause extra-intestinal complications related to the central nervous system (3−35%), pulmonary system (1−6%), cardiovascular system (1−5%), and hepatobiliary system (1−26%).[15] Owing to the excessive use of antibiotics, the rapid emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *S.* Typhi made *Salmonella* infection extremely difficult to treat.[16] To study the *in vivo* pathogenesis of *S.* Typhimurium, many susceptible mouse strains, such as BALB/c and C57BL/6, mimicking the symptoms of typhoid fever in humans are extensively used.[17,18] However, very few suitable models are available to study the *in vivo* pathogenesis and disease progression of *S.* Typhi. Additionally, a reduction in the size of its functional genome by either pseudogene formation or the absence of specific genes made our understanding of typhoid fever extremely difficult. Hence, a comprehensive discussion is required to explain the complexity of the interaction between

Received: April 9, 2023
Accepted: June 30, 2023
Published: July 14, 2023

ACS Publications

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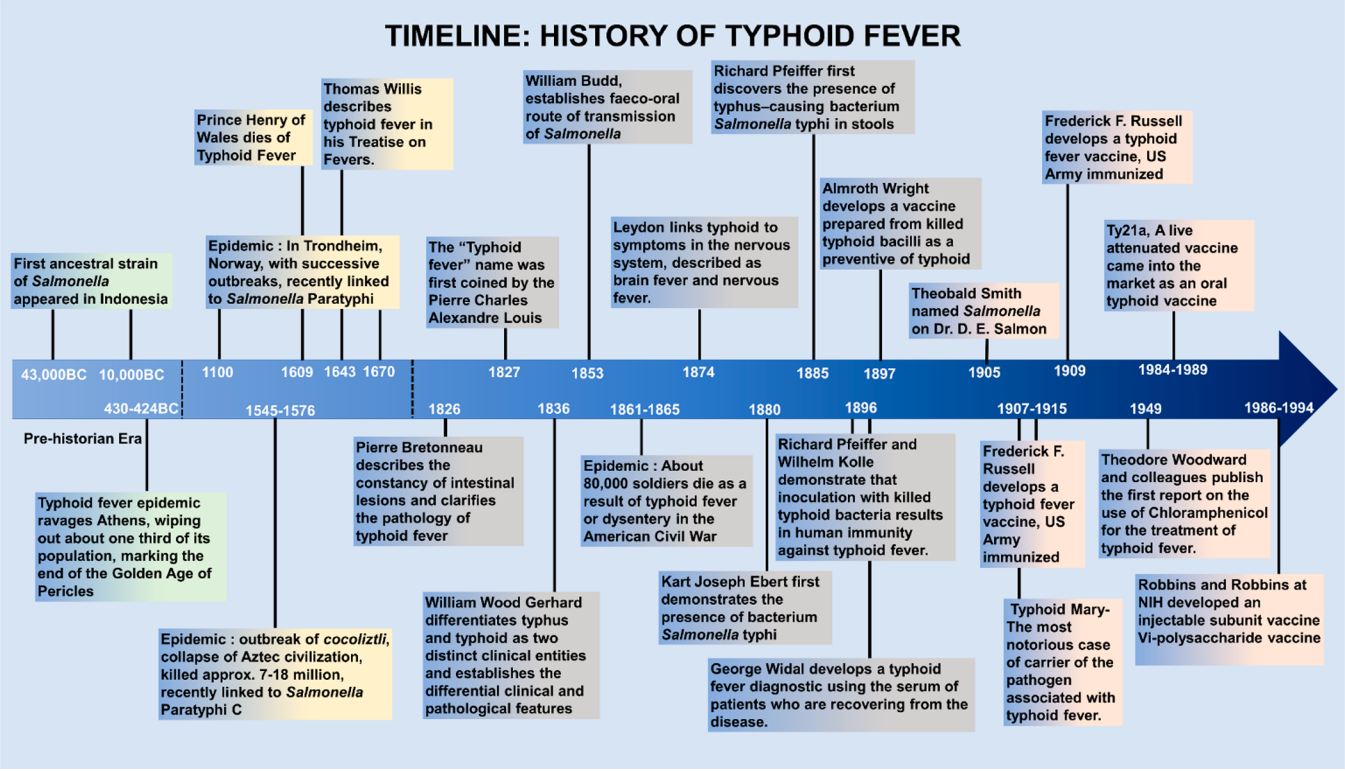

Figure 1. A timeline history of typhoid fever.

S. Typhi and the human host. This Review will revisit the history, global epidemiology, pathogenicity, virulence, and extra-intestinal complications related to typhoid fever and recent developments in these research domains.

HISTORY OF TYPHOID FEVER AND GLOBAL EPIDEMIOLOGY

History of Typhoid Fever. It is well established that *Salmonella* has existed and evolved on Earth with a long, multihued, and entrancing history of evolution. The name typhoid fever was derived from the Greek word "*τυφος*", which stands for smoke, stupor, and obscurity associated with the diseased person, as they experienced stupor and neuropsychiatric symptoms in severe infection. Even though the description of typhoid fever was first made as early as 1643 by Sir Thomas Willis, it was often confused with typhus. However, typhus is a flea-borne illness caused by *Rickettsia typhi* and *Rickettsia felis,* with symptoms ranging from fever, headache, rash, and hepatitis to diverse organ prognosis.[19] A brief timeline of the history of typhoid fever, therapeutic interventions, and vaccine development is depicted in Figure 1.

In the context of typhoid vaccine designs, Vi capsular polysaccharides (Vi CPS) require only a single administration dose and elicits low reactogenicity. However, Vi CPS is a T-independent type 2 (Ti2) antigen that cannot be immunogenic in infants. New and future generations of vaccines include Vi glycoconjugate, O-antigen glycoconjugate, recombinant proteins, and proteins isolated and purified from whole *Salmonella*. These vaccine development advancements have improved the protection against *Salmonella,* allowing low reactogenicity and better memory response.[20] As important as history is to understand this ancient pathogen, it is equally imperative to delve into the current global burden on healthcare and economies. Therefore, we will discuss global epidemiology in the next section.

Global Epidemiology. The overall burden of foodborne disease is enormous, accounting for 33 million deaths annually. Almost 1 in 10 people fall ill with foodborne illness, and *Salmonella* is one of the four predominant causes of diarrheal diseases globally. South-central Asia and Southeast Asia have the highest incidence of illness, followed by Latin America and Africa. In 2017, typhoid and paratyphoid fever alone accounted for 14.3 million illnesses globally. The same report suggested that *S.* Typhi caused around 76.3% of enteric fever, with a global fatality rate of 0.95% in 2017. Around 14.1% of total typhoidal cases are accounted for Southeast Asia, East Asia, and Oceania super-regions, followed by 12.1% of total typhoidal cases reported from sub-Saharan Africa.[21] The available general case reports on the prevalence of Typhoidal fever in sub-Saharan Africa and Latin America are limited. However, a recent systemic review of sub-Saharan African cases suggests that the incidence of occurrence in African countries is highly heterogeneous and increases with better surveillance of healthcare systems with time.[22] In Latin America, typhoid is more prevalent than paratyphoid.[21]

Typhoid and paratyphoid fevers are pervasive in young children (especially under the age of 5) and older adults in lower-income countries and are often life-threatening. Enteric fevers are endemic in developing countries, where adequate sanitation and acute clean water shortages hold paramount public health concerns.[23,24] Even though typhoid and paratyphoid clinical symptoms are indistinguishable, the epidemiology, susceptibility to antibiotics, and geographical distributions are distinct.[25,26] Paratyphoid to typhoid fever cases are higher in developed areas such as Central Europe (90.7%) and high-income subcontinents such as Australia, Asia Pacific, North America, and Western Europe. On the other hand,

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

Central Asia, Eastern Europe, Latin America, North Africa, Middle East, South Asia, Southeast Asia, East Asia, Oceania, and Sub-Saharan Africa account for approximately 53−98% of typhoid fever of the reported enteric fever cases.[21]

A study from Cambodia showed that approximately 42.9% of isolates of *S.* Typhi demonstrated MDR, whereas only 11.5% of *S.* Paratyphi isolates displayed increased resistance to ciprofloxacin, and none of the isolates of *S.* Paratyphi exhibited MDR.[26] Typhoidal and paratyphoid outbreaks in developed countries are usually linked to travel history to endemic areas.[27] In the past few years, the rate of *S.* Paratyphi A infection has surged, accounting for nearly 50% of all cases, especially in the Southeast Asian population.[24,28] A few studies also point out that genetic factors influence susceptibility to enteric fever. VAC14 is a phosphoinositide-regulating protein, and single nucleotide polymorphism (SNP) in VAC14 renders the host with an increased *S.* Typhi susceptibility. VAC14 SNP increases plasma membrane cholesterols, resulting in increased docking of *S.* Typhi to host cells.[29] Studies from Jakarta, Indonesia, demonstrate that the gastric mucus lining expresses cystic fibrosis transmembrane conductance regulator (CFTR), which has been shown to act as adherent and facilitate the entry of *S.* Typhi but not *S.* Typhimurium into intestinal epithelial cells.[30] Therefore, mutations in CFTR (such as F508del and IVS8CA) provide some protection against *S.* Typhi infection.[31] Extraintestinal salmonellosis is strongly associated with the immune states of individuals. Thus, it affects immune-compromised persons.[32,33]

SYMPTOMS AND DIAGNOSIS

The typhoidal and paratyphoidal fevers cannot be clinically differentiated. It takes about 8−14 days of incubation after infection. The incubation period also varies, with a range of 3−60 days. The disease can last from a few weeks to several months, depending upon the initial exposure to the infectious dose and the diagnosis and treatment time. These parameters also govern the subsequent severity of the disease and related complications. However, in NTS the infection is more localized, causing gastrointestinal inflammation. The incubation period for NTS is about 2−3 days, and the disease lasts for 3−7 days and is self-limiting in a healthy host without any underlying conditions.[34−36] NTS serovars develop severe complications in immune-compromised individuals, such as systemic secondary-site infection, chronic conditions (reactive arthritis and irritable bowel syndrome), and other febrile illnesses.[37] We have listed the common and differential typhoidal serovar and NTS infection symptoms for ease of interpretation in Table 1. Clinical diagnosis of typhoid involves a history of the patient's residence and travel (from endemic areas) and the presentation of febrile illness for more than 3 days with gastrointestinal manifestations (pain, constipation, or diarrhea). The diagnosis is difficult in the first week of disease onset, but recent advancements have made it easier. In the early 21st century, despite the medical advances in developing countries, laboratory diagnosis can be divided into conventional typhoidal diagnosis (bacteriological culture and Widal test) and advanced typhoidal diagnosis (polymerase chain reaction (PCR) based, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), hemagglutination, and coagglutination). In the subsequent subsections, we will discuss the currently available diagnoses for typhoidal fevers.

Table 1. Symptoms of Typhoid Fever and Non-Typhoidal *Salmonella* Infection (or Salmonellosis)

Salmonella enterica serovar	disease and symptoms
typhoidal (human-restricted) Typhi, Paratyphi, and Sendai	enteric fever, abdominal distention and pain, transient diarrhea or constipation, a maculopapular salmon-colored rash on the trunk
non-typhoidal (broad range) Typhimurium, Enteritidis, Dublin, Newport, et cetera.	gastro-intestinal, abdominal distention and pain, vomiting, inflammatory diarrhea

Bacterial Culture-Based Diagnosis. The gold standard diagnostic tool for typhoidal serovar and NTS diseases is still culturing stool, blood, and bone marrow samples. The choice of samples, such as body fluids or feces, depends on the type of infection diagnosed early; for example, stool samples are usually taken for NTS infection associated with localized inflammation. However, this technique may become less sensitive once the infection becomes systemic.[38] On the case of systemic enteric fevers such as typhoid and iNTS infection, blood or bone marrow is sampled during the first week of fever; later, urine or stool samples are evaluated. Getting culturable *Salmonella* in the first week of symptoms onset is particularly tricky. Thus, diagnostic laboratories perform the test multiple times, and with a large volume of blood the sensitivity of this diagnosis remains only about 40−80%.[39−41] For genus-level identification, manual methods or automated systems such as MALDI-ToF are used.[42] *S.* Typhi usually gives weak H_2S production and a negative ornithine decarboxylase upon biochemical aspect identification, whereas *S.* Paratyphi results in negative H_2S, citrate, and lysine reactions.[42]

Serotyping and Serological Diagnosis. *S. enterica*, being a multiform pathogen, has over 2600 serovars identified to date. Therefore, the serotyping of the clinical isolates plays a significant role in central public health repositories for endemic serovar identification, epidemiological analysis, surveillance, and disease outbreak studies. The *Salmonella* subtyping is conducted according to the White−Kauffman−Le Minor scheme. The conventional scheme of serotyping of *Salmonella* evaluates the expression of surface antigens such as somatic (O), flagellar (H), and capsular (Vi) antigens. However, the Vi antigen is exclusively present in *S.* Typhi, *S.* Paratyphi C, and *S.* Dublin.[43] Based on somatic antigen, *S. enterica* can be divided into 46 serogroups,[44] and there are 114 individual flagellar antigens, resulting in 2600 serovars.[45] The old serotyping methods were tedious and time-consuming for typing an isolate. Several new techniques aid the process of identification of *Salmonella sp.* serovars, such as pulsed-field gel electrophoresis (PFGE),[46,47] ribotyping,[48] clustered regularly interspaced short palindromic repeat (CRISPR),[49,50] and multilocus sequence typing (MLST).[51,52] The most performed serological diagnosis employs the Widal tube agglutination method. It is one of the primitive methods used for more than a century to diagnose typhoid or enteric fever. In this test, the killed *S.* Typhi and Paratyphi A bacteria are scored based on agglutinating antibodies to flagellar (H), LPS (O), and Vi antigens. However, the efficacy of this method is widely questionable due to its cross-reactivity with different serovars and low sensitivity.[53,54] Reports of cross-reactivities with unrelated diseases like malaria, dengue fever, and brucellosis in enteric fever-endemic areas are prevalent. Despite all of the drawbacks, it is still extensively used in areas lacking public health infrastructure, mainly because of its low cost and ease of use.

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ACS Omega 2023, 8, 25674−25697

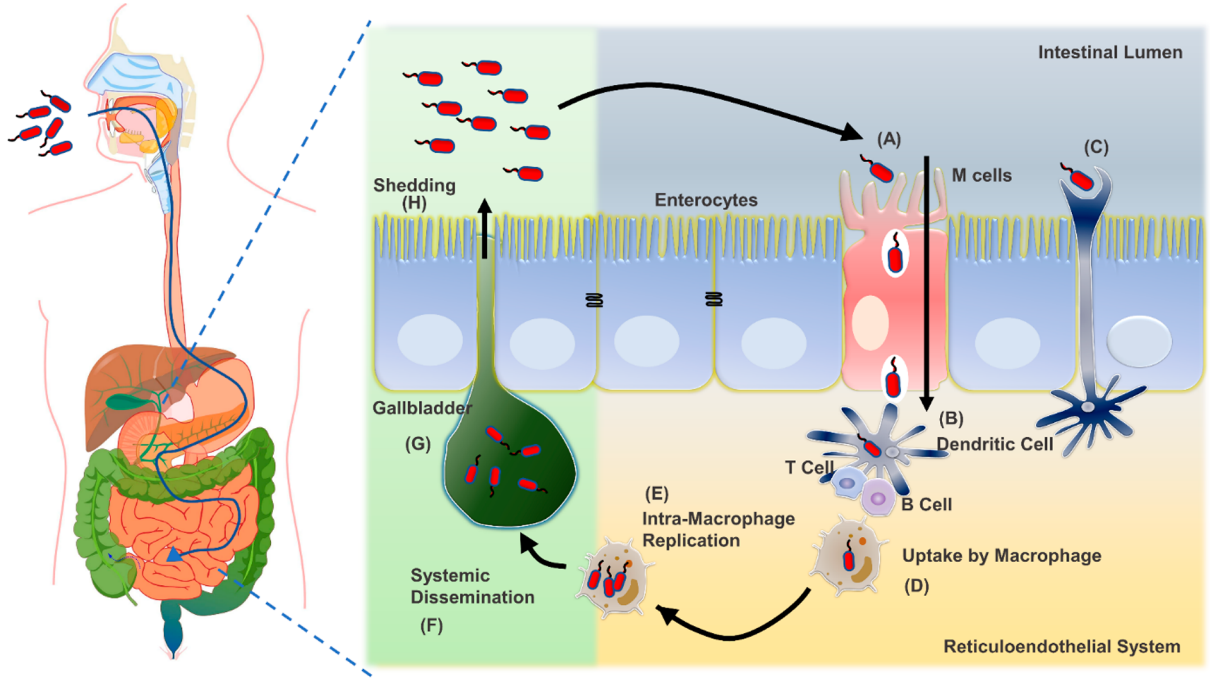

Figure 2. The course of *Salmonella* infection and long-term carriage. Initially, (A) *Salmonella* preferentially enters M cells, (B) which transport them to the lymphoid cells (T and B) in the underlying Peyer's patches. (C) It can also be taken up by dendritic cells, elongated dendrites through the intestinal epithelial barrier. (D) Once across the epithelium, *Salmonella* serotypes associated with systemic illness enter intestinal macrophages and (E) undergo intramacrophage replication and (F) disseminate throughout the reticuloendothelial system, leading to systemic infections. (G) Further colonization of the gallbladder leads to (H) chronic carriage and the bacterium's shedding through this mechanism. The green background shade depicts the GI tract near the gallbladder opening, and the yellow background depicts the GI tract's intestinal areas.

Based on a similar principle, some commercially available diagnostic tests adopt the detection and even quantification of antibodies in the patient's serum against several *Salmonella* antigens, such as outer membrane proteins or LPS. However, significant drawbacks remain, including low sensitivity (69%) and specificity (79%).[55,56] Hemagglutination tests are also routinely used in various countries. A report from India has shown that the anti-LPS HA test has a sensitivity of 60% and a specificity of 98.2%.[57] Similarly, 70% sensitivity and 92% specificity have been reported for the reverse-phase hemagglutination test (RPHA). These tests propose a convenient alternative for the Widal test to detect *S.* Typhi in endemic areas.[58] Since there is an increasing need for rapid, reliable, and cost-efficient testing for *Salmonella* to meet the need of the current situation, less sensitive detection of bacteria by PCR from blood samples is widely conducted.

Advanced Diagnostic Techniques. The routine bacteriological culture has a 100% specificity and about 54% sensitivity, and the PCR-based ones have about 98.1% specificity and 85.4% sensitivity[59] in samples from blood and bone marrow for the flagellin gene (*fliC*), Vi, O, and H antigens, *hilA* (an SPI-1 effector), and 23S rRNA, to name a few.[40,60−70] Another widely used technique is multiplex PCR, which directly identifies gastrointestinal pathogens in clinical stool samples. Although it can target and simultaneously amplify an array of genes,[71,72] the results still require further testing and validation through culturing for serotype classification. MALDI-TOF-based diagnosis is a newly emerging technique for reliable and rapid detection and typing of bacteria. Two of the FDA-approved MALDI-ToF systems for *Salmonella* detection are the Bruker Biotyper and bioMérieux Vitek MS systems. However, none of them can detect the serovar.[73] Next-generation sequencing (NGS) for diagnostics and bacterial typing using whole-genome sequencing (WGS) has been on the rise late on as well due to a drastic reduction of cost and a significant increase in downstream tools and bioinformatics analysis for sequence assembly and comparative genomics. Apart from the contig arrangements and analysis, WGS data also facilitate identifying SNP and provides us with single-nucleotide changes among *Salmonella* serovars. This approach enables the study of phylogenetic relationships and disease outbreak epidemiology.[74−76] WGS-based technologies are at the beginning of the exponential phase, and multiple technologies that have low cost, rapid, and reliability for clinical isolate typing are still in the pipeline, further leading to a shift in the current gold standard for clinical and public health sectors in phylogenetic relationship, origin of evolution, epidemiological, and geographical analyses of newly emerging and endemic serovars.

MODELS TO STUDY *SALMONELLA* INFECTION

S. Typhihas is adapted to be highly specific to human hosts. Therefore, there are very few *in vivo* models for studying the infection prognosis. *S.* Typhimurium infection and colonization in natural resistance-associated macrophage protein-negative (*Nramp1*$^{-/-}$) C57BL/6 or BALB/c mice induces typhoid fever-like symptoms and helps to unravel the infection biology of *Salmonella* Typhimurium.[77−79] Since typhoidal serovar and NTS share only 89% of the genes, the findings in *S.* Typhimurium are extrapolated to *S.* Typhi.[80] However, contradictory reports in the last two decades also indicate that, due to the loss of genes and pseudogenization in *S.* Typhi, all of the findings in *S.* Typhimurium cannot be directly applied to *S.* Typhi. In detail, we have discussed the loss of genes and

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

pseudogenization in *S.* Typhi in the section on virulence determinants of *Salmonella* pathogenesis. The humanized mouse model in a severely immunodeficient (*e.g.*, SCID or Rag2$^{-/-}$ γC $^{-/-}$) mouse with the human immune system is the nearest animal model to study *S.* Typhi infection. These mice are engrafted with CD34^{+} human umbilical cord blood stem cells, human fetal hematopoietic stem and progenitor cells, or human leukocytes.[81−84]

More recent research in *Salmonella* biology sheds light on the organoid or enteroid models, which help fill the gap between *in vivo* and *in vitro* research systems. C. A. Nickerson et al. was among the first groups to show a significant difference in *Salmonella* infection in 2D and 3D organoid culture models of Int407.[85] Two of the broad classifications of the 3D organoid model are mouse-derived and human-derived organoids. Zhang and colleagues performed one of the most primitive works with mouse-derived organoids. The observations revealed the bacteria-mediated tight junction disruption, NF-κB-induced inflammatory response, and downregulation of Lgr5 (stem marker). However, a faulty experimental setup led to infection from the basolateral side.[86] The intestinal enteroid model also provided an excellent model for studying different cell types regulations such as Paneth cell (PC) degranulation upon LPS interaction and the antimicrobial role of Paneth cell α-defensins.[87,88]

Human-derived models help to further the studies, as they carry the specific human genetic makeup instead of mouse models. Few *Salmonella* pathogenesis studies utilize adult stem cells (ASCs) and pluripotent stem cells (PSCs) derived from human-enteroid models. In 2018, K. P. Nickerson et al. introduced another human-derived enteroid model system using human tissue biopsies and human ileum. The transcriptional profiling of the bacteria and host tissue revealed that *S.* Typhi downregulated critical host genes that are responsible for bacterial clearance, host immunity, and cytoskeleton rearrangements. The silent phase of infection associated with *S.* Typhi is attributed to the downregulation of SPI-1, which minimizes intestinal inflammation.[89] Recent studies of *S.* Typhi in the *C. elegans* model system shed light on the usage of this system to answer research questions about *S.* Typhi research.[90] Enteroid, humanized mouse, and cell culture models will be instrumental in answering the multiple open-ended questions about *Salmonella.*

VIRULENCE DETERMINANTS OF *SALMONELLA* PATHOGENESIS

Unless otherwise stated, we aim to shed light upon *Salmonella* pathogenesis here, pertaining to typhoidal serovar and NTS. Upon ingestion of contaminated food and water, bacteria enter the digestive tract and overcome the acidic pH of the stomach with an excellent acid tolerance system called *phoPQ.* In the subsequent events, they reach the intestinal milieu, where they further escape several host-innate immune arsenals such as digestive enzymes, bile salts,[91] secretory IgA,[92,93] antimicrobial peptides (AMPs).[94−97] *Salmonella* then traverses the intestinal mucus layer to reach the epithelium beneath. In the small intestine, *Salmonella* is taken up preferentially by microfold (M) cells (Figure 2). The M cells are specialized cells that help sample food antigens from the intestinal lumen through pinocytosis. This sampling further channelizes the transfer of *Salmonella* to various immune cells in the Peyer's patches.[98,99] Alongside *Salmonella* inducing its uptake through bacterial-mediated endocytosis in nonphagocytic cells, *Salmonella*-mediated endocytosis into the enterocytes is initiated by the adhesion of bacteria to enterocytes by various molecules like fimbrial adhesins. Postadhesion, *Salmonella* can mediate its uptake by inducing membrane ruffling due to extensive modulation of the actin cytoskeleton of enterocytes.

On the other hand, CD18^{+} phagocytes can also phagocytose *Salmonella* from the intestinal lumen, thereby translocating it to Peyer's patches.[100] Once the bacteria reach the reticuloendothelial system, the macrophages can phagocytose the typhoidal serovar and NTS through phagocytosis and macropinocytosis. In the upcoming sections, we discuss the subsequent events about macrophages. The NTS elicits a strong Th1 immune response in immune-competent individuals due to pathogen-associated molecular patterns (PAMPs). It is characterized by IL-18-mediated recruitment of bone marrow-derived phagocytes, neutrophils, and B and T cells in the lamina propria, leading to phagocytosis of invading *Salmonella*. This response limits the dissemination of NTS from becoming a systemic infection, leading to self-limiting gastroenteritis.[101]

Upon entering the macrophages, *Salmonella* senses the phagosomal environment, activating various virulence arsenals to thrive and replicate in the macrophages in otherwise microbicidal niches and leading to dissemination throughout the reticuloendothelial system.[102−109] *S.* Typhi can surpass the host immune checkpoints, including lymph nodes that restrict the infection.[110] Induction of *Salmonella* pathogenicity island 1 (SPI-1) transcription and Vi-polysaccharide downregulates flagella to subvert the early inflammatory response in *S.* Typhi, one of its well-known PAMPs.[111,112] In *S.* Typhi, the Vi capsular antigen synthesis begins once the bacterium marks its entry into the ileum region of the gut.[113] *S.* Paratyphi A does not express Vi, yet it is capable of causing typhoid-like illness.[25] According to reports, S. Typhi lacking the Vi antigen is capable of causing infection,[114] indicating that Vi capsular polysaccharide is not entirely indispensable for the pathogenesis of typhoidal serovars.

The molecular mechanisms of *Salmonella* pathogenesis are highly complex and well-coordinated. Pathogenic strains of *Salmonella* spp. differ from their nonpathogenic relatives by harboring specific pathogenicity genes organized into clusters called pathogenicity islands.[115−117] Pathogenic islands are gene clusters incorporated into the genome, in either the chromosome or a plasmid, with variable GC content and codon usage compared to the surrounding regions. They are also flanked by direct repeats, insertion sequences, or tRNA genes, acting as sites for DNA recombination. Pathogenic strains of *Salmonella* have acquired these islands from other pathogenic bacteria during evolution by horizontal gene transfer. Some are conserved throughout the *Salmonella* genus, whereas others are exclusively found in specific serovars. To date, 24 SPIs have been identified, of which SPI-1 and SPI-2 have been vastly characterized. SPI-1 is required for the bacterium to invade the nonphagocytic epithelial cells, whereas SPI-2 is necessary for bacterial survival and replication inside the macrophages.[118] The remaining SPIs contribute to other pathogenic attributes like fimbrial expression, magnesium and iron uptake, multiple antibiotic resistance, intracellular survival, and systemic dissemination.[119]

S. Typhi is an example of reductive evolution where the serovar has undergone functional inactivation of genes (pseudogenization of 200−300 genes) to adapt specifically to the human host.[120] *S.* Typhi and *S.* Typhimurium have 11 SPIs

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

Table 2. Host Factors That Are Exploited by *Salmonella* Effectors to Facilitate Its Uptake into Non-Phagocytic Cells

host complex targeted	bacterial effectors	mechanism	refs
the WAVE regulatory complex (WRC)	SopB, SopE, SptP	the WRC complex is essential for the formation of lamellipodia, which is governed by Rac1 and Arf1 activity. GEF mimic SopE can activate Rac1, and Arf1 is activated by activity of SopB, which helps in the accumulation of PI (3,4,5) P3 and ARNO at the site of entry, leading to the recruitment of WRC and the promotion of the Arp2/3 complex for actin remodelling. SptP is responsible for the inactivation of Rac1 and returning the cell to the resting stage.	267−275
the WASH complex	not identified	the WASH (Wiskott−Aldrich syndrome protein and scar homologue) complex, also responsible for activating Arp 2/3-dependent actin polymerization, acts downstream of RhoA and accumulates at the *Salmonella* entry site.	270, 276, 277
myosin-mediated contractility	SopB	actin stress fibers are decorated with myosin IIA and IIB upon infection. SopB helps in hijacking and activating myosin II through the activation of RhoA and downstream Rho kinases (Rocks). the exact molecular mechanism remains elusive, but altered phosphoinositide dynamics might be involved.	128, 278
formins	SopB, SopE/E2	formins are a family of proteins that are nucleators of actin polymerization independently of Arp2/3. FHOD1 also lies downstream of RhoA and can be activated by Rac1. SPIRE1 (pathogen entry) and SPIRE2 (replicative niche) are also involved in cytoskeleton remodelling, as these help in nucleating the assembly/bundling of straight actin fiber.	130, 135, 279, 280
the exocyst complex	SipC, SopE/E2	Exo70 is part of a hetero-octameric exocyst complex that helps in vesicle transport, and it interacts with SipC. assembly of an exocyst complex requires activation of RelA by SopE/E2. the exact molecular mechanism for this complex-mediated entry also remains elusive, however, it is proposed that it may fulfill the extra phospholipids requirement during the formation of membrane ruffles.	281
IQGAP1	SopE/E2, SopB	IQGAP1 has a homologous domain of GTPase activating protein (GAP, inactivators of Rho GTPases), thus it binds GTPases (Rac1 and Cdc42) and prevents its inactivation. IQGAP1 also acts as a molecular scaffold for downstream MAPK/ERK signaling and various phosphoinositide kinases that might be in relation to SopB.	282−285
annexins	SopE/E2, SopB	annexins (A1, A2 and A5) are calcium-dependent membrane=binding proteins that indirectly regulate cytoskeleton membrane dynamics, and they accumulate at the site of *Salmonella*-mediated entry. A2 along with p11 protein binds AHNAK (a phosphoprotein regulator of membrane). exact molecular dynamics have yet to be elucidated.	286
myosin VI	SopE/E2, SopB	myosin VI plays a key role in endocytosis, and SopE specifically recruits it at the site of entry via Rac1-WRC and Rac1-p21activated kinase (PAK) pathway. myosin VI also recruits a PI3P-binding, actin-binding and GEF for Cdc42 called frabin at the site of membrane ruffles.	128, 272, 287−289
villin	SipA, SptP	villin, an actin-binding and severing protein, is required for *Salmonella* to enter into the polarized enterocytes. it is suggested that severing leads to the generation of barbed ends, which are essential for new filament growth. the activity of villin is regulated by SipA and SptP.	290

in common (1−6, 9, 11, 12, 13, and 16). SPI-8 and SPI-10 were initially found in *S.* Typhi and are considered absent in *S.* Typhimurium. There is only one SPI specific to *S.* Typhimurium, viz, SPI-14, and four SPIs specific to *S.* Typhi, viz., SPI-7, SPI-15, SPI-17, and SPI-18.[121] SPI-19, SPI-20, and SPI-21 are absent in both serovars (*S.* Typhi and *S.* Typhimurium).[122] SPI-7 and SPI-11 code for the Vi antigen and the typhoid toxin, two specific virulence determinants of *S.* Typhi.[123] A type IVB pilus encoded by SPI-7 is usually used by *S.* Typhi while adhering to human monocytes and epithelial cells by interacting with the CFTR receptor.[120] Other virulence determinants include the flagella, which activate innate immune response via the recognition of monomeric flagellin by the NLR family, apoptosis inhibitory proteins (NAIP), and TLR5 receptors.[123]

This section discusses how the coordinated expression and functioning of virulance determinants in *Salmonella* aid in its entry, survival, and dissemination in the host body. Research in nonphagocytic cells and *Salmonella* interaction has focused chiefly on *S.* Typhimurium, even in cell culture models. Therefore, in the upcoming section with nonphagocytic cells, most of the work is on *S.* Typhimurium until otherwise stated.

Interaction of *Salmonella* with Nonphagocytic Cells. Much literature suggests that entry into the nonphagocytic cells holds immense importance in disease manifestations, since the primary site of infection resides in the intestinal epithelial cells. Therefore, we have elaborately discussed the current knowledge of the pathogenesis of *S.* Typhimurium in nonphagocytic cells.

SPI-1 Facilitates Entry into Nonphagocytic Cells. In 1989, Galan and Curtiss discovered that the genes responsible for mediating entry into the nonphagocytic cells are encoded by SPI-1.[124,125] SPI-1 encodes a molecular syringe capable of injecting an array of bacterial effector proteins directly into the host cells. These effectors modulate host cell signaling (especially inflammatory ones) and induce the uptake of the bacteria into nonphagocytic cells. The general theory is that SPI-1 type 3 secretion system (T3SS) mediated uptake of *Salmonella* into the nonphagocytic host cells occurs through the injection of bacterial effectors, which are responsible for the rearrangement of the cytoskeleton resulting in the formation of membrane ruffles, a process similar to macropinocytosis. SopB, SopE, and SopE2 are critical determinants for the uptake of *Salmonella* in epithelial cell uptake, and combined mutation in all three genes shows defects in entry.[126] SopB is an inositol phosphate phosphatase that acts on phosphatidylinositol species to remove 4′- and 5′-phosphates, which is essential for membrane ruffling.[127] There is a SopB-dependent increase in phosphatidylinositol-3-phosphate (PI3P), phosphatidylinositol-3,4-bisphosphate (PI(3,4)P2) and phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P3) in infected cultured cells and on the SCV membrane.[128,129] Accumulation of these PIP lipids species leads to the recruitment of several host factors, such as guanine nucleotide exchange factors (GEFs), that further activate an essential Rho GTPase.[130] SopB also activates Akt or protein kinase B (PKB)-YAP, which facilitates *S.* Typhimurium's growth inside host cells and inhibits apoptosis.[131,132] The activity of SopB assisted by Vps34 and Rab5 on a *Salmonella*-containing vacuole (SCV) membrane leads to increased charge on the membrane, leading to lysosomal fusion inhibition conferring protection to SCV from acid hydrolases of the lysosome.[128] SopB also reduces the overall lysosomal numbers, giving the pathogen an upper hand compared to the host.[129]

On the other hand, *Salmonella* effectors such as SopE2 and SopE mimic host GEFs that activate Rho GTPase Cdc42,[133] and SopE can also activate Rac1.[134,135] Host Rho GTPase is significant in bacterial uptake by nonphagocytic cells such as

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

enterocytes and epithelial cells in the gallbladder. In conjunction with these effectors, SPI-1-encoded effectors such as SipA and SipC also facilitate uptake by directly interacting with actin-binding proteins. SipC is a part of SPI-1 translocon and can nucleate actin filament polymerization and bundle pre-existing actin.[136−138] SipA initiates actin nucleation and the bundling activity of SipC by the high-affinity binding and stabilization of actin filaments. Further, SipA also prevents depolymerization by host ADF and cofilin proteins.[139,140] However, SipA deletion mutants only have reduced invasion capacity, suggesting that SipA is not entirely indispensable but does facilitate the entry of the bacteria.[141] In contrast, a recent report challenges the existing model of ruffle-mediated entry into the cells. The report suggests that entry into the mouse gut epithelium occurs through the "discrete-invasion" model. This discrete invasion is facilitated by SipA, which is capable of manifesting significant elongation of local microvilli, and does not favor cooperative invasion.[142] SipB, SipC, and SipD are essential in forming the needle complex. Besides this, they have other functions, *e.g.*, SipB is necessary for *Salmonella*-induced caspase-1-dependent apoptosis and the release of IL-18[143−145] and SipC interacts with F-actin, which helps in pathogen internalization.[138] A potential functionality difference might appear between the typhoidal and nontyphoidal serovars due to amino acid substitutions in the SipD translocon of *S.* Typhi.[146] Although these effectors together orchestrate the uptake of the bacteria, several redundant pathways of the host are stealthily exploited by *Salmonella* for its benefit and to gain access to nonphagocytic cells (Table 2).

The invasion may also be influenced by bile, Mg^{2+}, and short-chain fatty acids.[147] The liver produces bile continuously and helps in the digestion and absorption of fats. Before its release into the intestines, bile is stored in the gall bladder at high concentrations. It is an essential environmental cue to upregulate virulence gene expression during infection within the host's gastrointestinal tract. *Salmonella* regulates the production of virulence factors following bile exposure. BarA is the sensor kinase that responds to environmental stimuli and acts through its cognate response regulator SirA to induce the expression of invasion genes. This pathway includes the induction of *hilA* expression and a *hilA*-independent effect on *invF* expression.[148] Bile represses the invasion capability of *S.* Typhimurium via BarA/SirA,[149,150] and *in vitro* studies have shown that bile exposure increased the invasive capability of *S.* Typhi in HeLa cells. A protein stability assay reveals that this observation is attributed to the more excellent stability of one of the chief regulators of SPI-1, HilD, upon bile exposure in *S.* Typhi.[151]

The temperature differentially regulates the expression of T3SS in typhoidal and nontyphoidal serovars. During fever, an increase in body temperature (39−42 °C) in an infected host reduces the invasion and motility in typhoidal serovars. SPI-2 also has a temperature-dependent regulation. Higher temperatures (42 °C) increased SPI-2 expression in both *S.* Paratyphi A and *S.* Typhimurium, although a significant increase in SPI-2-mediated intracellular replication was found only in *S.* Paratyphi A.[152] This is another example of adaptation, since fever marks the beginning of systemic dissemination of typhoidal serovars, where intracellular survival is more important than invasion.[125]

The Dual Lifestyle of Salmonella in Nonphagocytic Cells. After entering the epithelial cells, the pathogen can lead a dual lifestyle, either cytosolic or vacuolar. *Salmonella* remains encapsulated within a host membrane compartment, SCV, or it might quit the vacuole to thrive in the host cytosol. *Salmonella* modulates the intricate pathways of host endosomal−lysosomal maturation with SPI-2 effectors to establish a successful replicative niche. Once inside the cell, there is the downregulation of the expression of SPI-1 and the upregulation of another molecular syringe SPI-2-encoded T3SS, which spans the vacuolar membrane and bacterial membrane and can inject several effectors from the bacterial cytosol to the host cytosol. The central cues for the induction of SPI-2 are limited nutrients, low pH, antimicrobial peptide accumulation in the vacuolar lumen, and decreased Mg^{2+} concentration.[153] The pH sensing mechanism by intracellular *Salmonella* is mediated by a protein complex comprising SsaM, SpiC, and SsaL encoded by SPI-2. This complex can sense low and neutral pH and regulate effector translocation.[154]

A recent review on the genetic tuning of *S. enterica* describes all the aspects involving genetic switches of *Salmonella* to thrive in hostile host niches.[155] SPI-2 injects as many as 43 different effector proteins into the host cytoplasm, assisting in SCV maturation and establishing a replicative niche.[156−158] The SCV maturation is comprised of three different stages: early (<30 min postinfection), intermediate (30 min to 5 h postinfection), and late (>5 h postinfection). Each stage is distinct regarding bacterial and host proteins recruited on SCVs. Apart from mediating entry, SopB also serves a highly vital role in the initial biogenesis of SCV: it alters the phosphoinositide dynamics of the SCV membrane through hydrolysis of PI(3,5)P2 and PI(3,4,5)P3 to PI(3)P.[159−161] It is also responsible for recruiting GTPases, Rab5, and PI(3) kinase Vps34, which induces rapid remodelling of the membrane through early endosome antigen 1 (EEA1) mediated fusions with early endosomes within as little as 5 min of entry.[128,160,162] Recently, it was reported that SopB triggers rapid recruitment of cytosolic sorting nexin 18 (SNX18; an SH3-PX-BAR domain sorting nexin protein) that further assists SopB in its phosphoinositide phosphatase activity.[163] Next, the SCV membrane acquires several endocytic host protein markers, such as transferrin receptors (TfnR), Rab4, and Rab11,[164,165] which act as protective shelters in an otherwise hostile host environment.

Salmonella-Containing Vacuole (SCV) Maturation and Replication. The SCV maturation mimics host endocytic pathways by shedding off early, sorting, and recycling membrane markers within 30 min postinfection and acquiring a set of late endocytic marker proteins such as vacuolar-ATPase, Rab7, Rab9, lysosomal glycoproteins (LAMP1, LAMP2, and LAMP3/CD63), and RILP (Rab-interacting lysosomal protein).[166−170] Rab7 is the primary small GTPase acting in late endosomal and lysosomal vesicles, and Rab7 facilitates the recruitment of lysosomal glycoproteins, RILP, FYVE (another Rab-interacting lysosomal protein), and coiled-coil domain-containing protein 1 (FYCO-1).[171−173] Rab7 and its effector RILP recruit the motor protein dynein to SCV to initiate its centripetal movement, resulting in peri-Golgi localization of SCV. However, it is very compelling that *Salmonella* selectively excludes a few late endocytic markers such as mannose-6-phosphate receptors (M6PR), lysobisphosphatidic acid (LBPA), and lysosomal hydrolases (cathepsins) from the SCV membrane in the process of SCV maturation.[166,167,174,175]

S. enterica forms a highly complex stabilized network of tubules called *Salmonella*-induced tubules (SITs). Some of the

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

Table 3. *Salmonella* Pathogenicity Islands (SPIs), Genomic Structure, and Functions in *S.* Typhi

SPI	effectors	known function	(modified) function in *S.* Typhi	refs
SPI-3 (17-kb pathogenicity island located at 82 min, just behind the *selC* tRNA locus of *Salmonella*)	in the *mgtCB* operon, the gene *mgtC* is specific for *Salmonella*. *mgtB* which is a Mg^{2+} transporting ATPase. Another Mg^{2+} transporter coded by SPI-3 is *mgtA*. SPI-3. It also harbors the *marT-fidL* operon, encoding MarT transcriptional regulator and a hypothetical protein FidL	in the *mgtCB* operon, the gene *mgtC* is specific for *Salmonella*, facilitates intramacrophage survival, growth in low Mg^{2+} media, and survival in acidic environments of endosomes resulting from SPI-1-mediated invasion. MisL, coded by SPI-3, is an extracellular matrix adhesin involved in intestinal colonization	the region adjacent to the *selC* tRNA region hosts the maximum amount of variations between *S.* Typhi and *S.* Typhimurium along with some pseudogenes like STY4024 (*cigR*), STY4027 (*marT*), STY4030 (*misL*), STY4034, STY4035, and STY4037. a few more pseudogenes are also noted in other regions of SPI-3 like STY4012, STY4007, and STY4003. in brief, MisL, its regulator MarT, and an unknown putative transcriptional regulator (STY4012) are inactivated in *S.* Typhi. expression of *S.* Typhimurium *marT-fidL* in *S.* Typhi negatively affected the survival of the bacterium inside macrophage cells but not epithelial cells	291−295
SPI-4 (24 kb fragment located next to a potential tRNA-like gene at centisome 92)	SPI-4 encodes a type 1 secretion system (*siiCDF*), which encodes the almost 600 kDa protein (encoded by SiiE)	SiiE is a nonfimbrial adhesin protein, 600 kDa in size, containing 53 repeats of Ig domains. SiiE is secreted into the culture medium and mediates contact-dependent adhesion to the epithelial cell surface	SPI-4 has role in the intracellular uptake and survival in macrophages for *S.* Typhi. SiiE is coded by only one ORF in *S.* Typhimurium (STM4261), unlike in *S.* Typhi, where it is segmented into two ORFs in *S.* Typhi (STY4458 and STY4459) due to the presence of a stop codon. this implies that *siiE* is a pseudogene in *S.* Typhi that correlates with the loss of function of adhesin responsible for intestinal colonization by *S.* Typhimurium	237, 296, 297
SPI-5 (approximately 7.6 kb long and has been found next to *serT* tRNA)	encodes the effector proteins for both the T3SS system of SPI-1 and SPI-2	SopB translocated by SPI-1 T3SS helps in membrane ruffling and invasion. PipA contributes in the development of systemic infection. PipB translocated by SPI-2-encoded T3SS facilitates accumulation of lipid rafts and helps in intramacrophage survival	no difference has been observed between the two serovars apart from an additional ORF (STY114) that putatively encodes a transposase in *S.* Typhi	117, 298, 299
SPI-6 (located next to *aspV* tRNA gene at centisome 47 and it extends for 47 kb and 59 kb in both *S.* Typhi and *S.* Typhimurium)	SPI-6 has the *saf* gene and the *pagN* gene	the *saf* gene codes for fimbriae and the *pagN* gene codes for the invasion protein in both the serovars	a fragment 10 kb in size is present downstream of the *saf* operon only in *S.* Typhi, which includes putative transposase remnants (STY0343 and STY0344, both of which are pseudogenes), the fimbrial operon *tcfABCD*, and genes *tinR* (STY0349) and *tioA* (STY0350). although the T6SS gene cluster is intact in *S.* Typhi, the protein complex is thought to be nonfunctional due to the presence of a pseudogene form of SciI (VipB homologue). Wang et al. have disproved this assumption and have identified the genes *sciA*, *sciG*, *sciS*, and *vrgS* as important for T6SS function	299, 300
SPI-7 (largest SPI, 133 kb, located between two partially duplicated copies of the tRNA-*pheU* gene and contains about 150 genes)	encodes the capsular polysaccharides or the Vi antigen. it contains the *pil* gene cluster along with some of the genes of the conjugative plasmid like *tra* and *sam*	the *pil* gene cluster that is responsible for coding many virulence factors along with the SopE prophage (ST44) that encodes the SPI-1 effector SopE	the Vi capsule also acts to inhibit flagellin expression, causes limited recognition of *Salmonella* by NAIP, and helps to reduce levels of pyroptosis and IL-1β secretion of macrophages. Along with this, Vi has been reported to bind to cell surface, thus dampening the inflammation caused by MAPK signaling or IL-8 production	301−303
SPI-8 (6.8 kb and has been next to *pheV* tRNA gene that is found next to SPI-13)			the exact function of SPI-8 is yet to be elucidated but it is known to code for putative virulence factors	120, 304
SPI-9	encodes for virulence factors of the type I secretion system and encodes RTX-like protein. It shares a 40% nucleotide homology to *siiCDEF* genes from SPI-4		arranged in an operon whose promoter was upregulated in low pH and high osmolarity conditions in a RpoS-dependent fashion. All the proteins encoded by this pathogenicity island localize in the membrane faction, supporting their putative role as T1SS. SPI-9 contributed to the adherence of *S.* Typhi to epithelial cells under high osmolarity or low pH or in situation mimicking conditions of the small intestine	80, 298, 305
SPI-10 (32.8 kb, next to *leuX* tRNA gene at centisome 93)	In *S.* Typhimurium, it is substituted by a 20 kb uncharacterized island without any SPI annotation that comprises functionally unrelated genes sharing little homology to sequences from the genomic database	there is possible relationship of this sequence with DNA repair, deletion of this island in *S.* Typhimurium 14028 attenuated the virulence of the strain	a full P4-related phage named ST46 that harbors *prpZ* as cargo genes responsible for encoding the Ser-Thr protein involved in *S.* Typhi survival in macrophages. many other pseudogenes are also found in *S.* Typhi, namely, STY4835 (IS*1230*), STY4836 (*sefA*), STY4839 (*sefD*), STY4841 (*sefR*), STY4845 (a thiol−disulfide interchange protein), and STY4848 (putative transposase). the homologous ORFs STY4842−4846 of *S.* Typhi includes the *srgA* gene. The *srgA* gene encodes a functional disulfide oxidoreductase in *S.* Typhimurium unlike that in *S.* Typhi, where it is a pseudogene (STY4845). it contributes to virulence factors that lead to the formation of Sef fimbriae, a phenomenon restricted to *S.* Typhi and *S.* Enteritidis. The role of cryptic bacteriophage is yet to be elucidated	304, 306, 307
SPI-11 (6.7 kb in *S.* Typhimurium and 10 kb in *S.* Typhi)			*S.* Typhi lacks the putative envelope lipoprotein *envF*, but it retains six additional ORFs (STY1884−1891), including the typhoid toxin CdtB. the CdtB toxin is elaborated in the section Interaction of Salmonella Typhi with Phagocytes	308
SPI-12 (is located next to *proL* tRNA centisome 48, 6.3 kb long in *S.* Typhi)		SPI-12 is responsible for causing systemic infection in mice for *S.* Typhimurium 14028	in *S.* Typhi, three ORFs are pseudogenes, viz., STY2466a, STY2468, and STY2469. Only the *sspH2* gene is functional in this island	146, 309

https://doi.org/10.1021/acsomega.3c02386
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Table 3. continued

SPI	effectors	known function	(modified) function in *S.* Typhi	refs
SPI-13 (next to the *pheV* tRNA gene at centisome 67 in *S.* Typhimurium and *S.* Typhi.; there is an 8 kb fragment different in the serovars, which corresponds to SPI-8 in *S.* Typhi)	In *S.* Typhimurium, the ORFs STM3117−3123 code for virulence-associated genes	the genes are involved in intracellular replication in murine macrophages and contribute to systemic infection of mice	*S.* Typhi SPI-8 harbors two bacteriocin immunity proteins (STY3281 and STY3283) and four pseudogenes. The virulence properties of SPI-8 are unknown. The conserved 17 kb in SPI-13 has not been found to contribute to virulence	6, 310, 311
SPI-14		the function of this SPI has yet to be understood	although these are found in *S.* Enteritidis and *S.* Typhimurium, they are absent from *S.* Typhi and *S.* Paratyphi A	304
SPI-15 (6.5 kb island that is present near the *glyU* tRNA gene in *S.* Typhi)		absent in *S.* Typhimurium	constitutes five islands encoding hypothetical proteins, which have been identified by bioinformatic analysis	312
SPI-16 (4.5 kb fragment located next to *argU* tRNA)	five ORFs in *S.* Typhimurium		seven ORFs in *S.* Typhi. Four of these genes are pseudogenes in *S.* Typhi. the three remaining ORFs in *S.* Typhi have a high level of similarity with P22 phage involved in seroconversion. they are suggested to cause O-antigen glycosylation and cell surface variation.	312, 313
SPI-17 (inserted next to an *argW* tRNA site)	this pathogenicity island contains seroconversion genes homologous to P22 phage	it showed a high similarity to genes of SPI-16, including a putative lipopolysaccharide modification acyltransferase	most of these genes are pseudogenes in *S.* Typhi	312
SPI-18	This genomic island is missing in *S.* Typhimurium		codes for two putative genes. One of these is *hlyE*, encoding a hemolysin related to the *Escherichia coli* K12 HlyE hemolysin. Fuentes et al. have shown that *S.* Typhi *hlyE* mutants are impaired in their capability to invade human epithelial cells in vitro, and its heterologous expression in *S.* Typhimurium has been seen to improve the colonization of deep organs in mice. additionally, a secreted 27 kDa invasin, coded by *taiA* (STY1499), increases bacterial uptake by human macrophages	304, 314, 315

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

well-studied SITs are *Salmonella*-induced filaments (SIFs). SIF formation occurs mainly in epithelial cells, and SIFs are the only SITs harboring LAMPs. *Salmonella*-induced filament A (SifA) is responsible for forming SIFs, and the SifA mutant cannot induce SIFs. The host protein that interacts with the SPI2 effector SifA is the SifA- and kinesin-interacting protein (SKIP, aka PLEKHM2). The association of SifA-SKIP inhibits the recruitment of M6PR by sequestering Rab9, which is essential for the maturation of endocytic vesicles. The reduced M6PR onto the SCV membrane leads to reduced delivery of lysosomal enzymes inside the SCV, thereby conferring protection from various host degradative enzymes.[176] SopD2, another SPI-2 effector, disrupts the Rab7-mediated recruitment of RILP and FYCO1 at the late stage of SCV maturation, leading to the shedding of RILP and FYCO1.[172,173,177] SseF and SseG form a tethering complex along with the Golgi-associated protein ACBD3.[178,179] SIFs also have access to the host cell's endocytosed content,[180−182] suggesting that SIFs enable nutrient acquisition. Long filamentous structures are potent in providing larger surface areas for more nutrient uptake and promoting bacterial survival and, more importantly, replication along with SCV. A recent study by Hensel and colleagues demonstrated a continuum between the SIFs and SCVs.[183] Moreover, auxotrophic mutants, incapable of inducing SIFs, cannot access the host nutrients.[184] There is a dearth of research on the exact molecular mechanism of *Salmonella* replication inside the host cellular niche. However, *Salmonella* perturbs the SCV and the lysosome ratio to successfully thrive inside the host niche by replicating with the SCV to maintain one bacterium per vacuole. The requirement of a dedicated vacuolar compartment per bacterium in the host cell increases the vacuolar load. Simultaneously, the SCV remains nonbulky, which helps avoid lysosomal fusion.[185] About 10−20% of intracellular bacteria quit their vacuolar niche to enter the cytosol.[186−188] About half of the SCVs are essential targets for host xenophagy and are subsequently eliminated, and the other half escape this by hyper-replication.[179,189] The cytosolic bacterial population triggers host cell pyroptosis and extrusion of enterocytes from the intestinal lumen by inducing a local burst. The cytosolic lifestyle gives *Salmonella* an advantage over the host, as hyper-replication in the enterocytes causes localized inflammation, and the bacteria can silently replicate in the vacuolar niche and remain hidden from host immune arsenals.

Salmonella Modulates Host Endocytic Protein. Apart from these host factors, another set of host proteins plays a role in SCV maturation and biogenesis, called soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins. SNAREs are the proteins that help endocytic vesicular fusion and fission and serve an intricate function in maintaining host endolysosomal pathway homeostasis. Newer insights into the role of SNAREs in SCV biogenesis suggest that *Salmonella* also modulates the host endolysosomal pathways to facilitate its survival and replicative niche. A recent report suggests that a host SNARE called syntaxin 8 interacts with SipA (SipA mimics cognate SNARE of syntaxin 8) to promote its fusion to early endosomes, thereby recruiting other syntaxins such as syntaxin 7 and syntaxin 13 to the SCV membrane, which potentially arrest its maturation and further assist in survival.[190] The SCV and infection-associated macropinosomes (IAMs) fuse with the help of SNARE proteins (SNAP25 and STX4) to maintain dynamic SCV growth and shrinkage.[191] SPI-2 mediates the SCV division in a syntaxin 3-dependent manner to facilitate *Salmonella* survival.[192] SipC recruits syntaxin 6, which helps acquire LAMP1 on SCV from the Golgi apparatus.[193] *Salmonella* can partially trigger autophagy in epithelial cells via SPI-1-encoded T3SS-1-mediated damage to the SCV membrane, exposing the SCV lumen to various host cytoplasmic ubiquitin ligases. Apart from this, the damaged SCV can also be recognized by galectin 8, which is capable of recruiting autophagic machinery.[194] SCV damage also leads to the release of *Salmonella* in the cytosol, which the ubiquitin systems of host cells can identify. Subsequently, cytosolic *Salmonella* leads to the bacteria's polyubiquitination, which is recognized by NDP52, OPTN, and p62 adaptor molecules linked to LC3B autophagic capture.[171] In Figure 3, we have described the necessary steps involved in SCV biogenesis, maturation, and interaction with host−endosomal−lysosomal pathways. However, it is interesting that if the SCV membrane remains undamaged, *Salmonella* can subvert the host autophagic machinery and thrive inside the host macrophages by employing SopB-mediated mechanisms.[195]

Interaction of *Salmonella* with Phagocytes. The phagocytes engulf *Salmonella* by a process called phagocytosis and initiate killing by mounting an antimicrobial response in the form of reactive oxygen species (ROS), reactive nitrogen species (RNS), cationic antimicrobial peptides (CAMPs), metal ion starvation, and acid stress.[196] Professional antigen-presenting cells such as dendritic cells and macrophages can prime $CD4^+$/ $CD8^+$ T lymphocytes by presenting bacterial antigens via major histocompatibility complexes (MHC) I and II. IL-18 influences the activation of $CD4^+$ T lymphocytes, resulting in the secretion of IFN-γ and the clearance of *S.* Typhimurium infection from the spleen of mice in a MHC-II-independent manner.[197] $CD8^+$ T lymphocytes are essential in clearing the infection caused by cytosolic bacteria such as *Listeria monocytogenes*.[198,199] However, the same is unknown during the infection of vacuolar pathogens such as *S.* Typhimurium.[200,201] The successful onset of typhoid fever in humans by *S.* Typhi depends on its ability to evade and eventually establish an intracellular proliferating niche in phagocytes. In the following part of this Review, we discuss the intelligent approaches employed by *S.* Typhi and *S.* Typhimurium to deceive the immune system and initiate systemic infections in humans and mice.

Intracellular Life of *Salmonella* in the Macrophage. The virulence of *S.* Typhi is unique in comparison with that of *S.* Typhimurium, which is capable of thriving inside a wide range of host cells, including murine macrophages (RAW264.7 and J774A.1 cells), human monocyte-derived macrophages (THP-1 and U937 cells),[202−204] dendritic cells,[205,206] and B lymphocytes.[207−209] To escape the immune response, invasive strains of *Salmonella,* such as *S.* Typhi Ty2 and *S.* Typhimurium D23580, downregulate their metabolic activity and start consuming lipids over carbohydrates.[210] After entering macrophages, *S.* Typhimurium stays inside modified membrane-bound SCVs.[211,212] The mature SCV recruits several proteins like LAMP-1 and Rab7 and inhibits their fusion with the lysosome, similar to nonphagocytic cells.[185,211,212] The presence of the SCV membrane protects the bacteria from intracellular threats such as ROS, RNS, CAMPs, etc.[213,214] Unlike the nontyphoidal serovars, *S.* Typhi prefers to invade and survive only inside human monocyte-derived macrophages.[215,216]

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

Table 4. Extra-Intestinal Infections by *S. Typhi*

organ infected	complications	symptoms	pre-disposing factors	diagnosis	treatment	refs
brain	"typhoid encephalopathy" under severe conditions, Parkinson's syndrome, motor-neuron disease, transient amnesia, symmetrical sensory-motor neurotrophy, schizophreniform psychosis, and Guillain-Barre syndrome	altered consciousness, delirium, and confusion along with other rare neurological symptoms like myelitis, altered sleep pattern, meningitis, and acute psychosis		neuroimaging with magnetic resonance imaging (MRI), angiography, computed tomography (CT), and/or radionuclide scans might be handy for diagnosis of brain abscesses, epidural empyemas, and subdural empyemas		316−317318319
cardiac impairments	major complications like (i) myocarditis and (ii) endocarditis to pericarditis and arteritis as rare complications	(i) ,icrocirculatory disturbances, edema, inflammation of the heart intramural vessels, lymphocytic, and macrophage infiltration of the stroma, sometimes with the formation of granulomas, and dystrophic and necrotic changes	underlying cardiac complexities like heart valvular abnormalities, congenital heart disease, or rheumatic heart disease	(i) abnormal doppler signal, ECG, CMR, indicators of cardiac injury (CK, AST, and LDH), electromyocardial biopsy (EMB). (ii) culturing the pathogen from the blood. transthoracic or transesophageal echocardiography might reveal the presence of vegetation	antibiotics and supportive management.	320, 321
lung disease	bronchopneumonia, lobar pneumonia, pleurisy with effusion, and empyema	fever, chills, coughs (with or without sputum), pleuritic pain, coarse crackles and bronchial breathing on auscultation, diarrhea, and leukopenia	prior abnormalities of lung or pleura with the presence of lung malignancies in some cases. mortality is usually high in geriatric patients, with underlying malignancies and immunosuppression due to antineoplastic treatments	chest radiographs or by isolation of the bacterium from sputum, protected specimen brush (PSB) material of bronchial secretions	*Salmonella* pneumonia calls for at least 2 weeks of antimicrobial therapy. under unresponsive antibiotic therapy, lobectomy might be an option	322−324
liver	hepatitis	high fever, relative bradycardia, and left shift of WBCs		liver damage markers like elevated serum alanine and aspartate transaminase levels, alkaline phosphatase levels, and γ-glutamyl transferase levels. abdominal ultrasonography (USG) or MR cholangiography. blood culture and serological tests for *Salmonella*		325
spleen		fever, chills, tenderness in the left upper quadrant, and splenomegaly	hemoglobinopathies, impaired host resistance, subacute bacterial endocarditis, IV drug abuse, trauma, diabetes mellitus, skin sepsis, respiratory tract infections, immunodeficiency, and urinary tract infections	ultrasonography (USG), USG-guided aspiration followed by culturing the pathogen, Widal test, stool culture, and blood culture	drainage of the largest abscesses along with antibiotic regimen is the favorable treatment. percutaneous drainage preferred. Patients with multiple abscesses unresponsive to percutaneous drainage might call for a splenectomy	326, 327
urinary tract	abscesses in kidney and nephrons. rare complications are IgA nephropathy, hemolytic-uremic syndrome, acute tubular necrosis, and Henoch-Schpnlein purpura	structural or functional abnormalities, pyelonephritis, dermoid cyst, calculi, dermoid cyst, and renal transplant		diagnosis is possible by culturing the pus from the abscesses	needs the proper antimicrobial regime. in the case of a perinephric abscess, a drainage procedure might also be required in addition to the administration of antibiotics	328
genital tract	pelvic inflammatory diseases (PID). along with the pelvis, *Salmonella* can form abscesses in pelvic bones and intraperitoneal spaces	fever, lower abdominal pain, and leukocytosis	most of the cases had underlying structural deformities like ovarian or dermoid cysts or were immunocompromised	increase in WBCs, ultrasonography. laparoscopic evaluation of the pelvic organs is the ideal method for diagnosing tubo-ovarian abscesses. isolation of *Salmonella* Typhi from purulent abscesses may make definitive diagnosis possible	7−14 days of antibiotic treatment in the case of genitourinary infections or longer if underlying complications as stone or abscess collection are seen	329, 330

https://doi.org/10.1021/acsomega.3c02386
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Table 4. continued

organ infected	complications	symptoms	pre-disposing factors	diagnosis	treatment	refs
musculoskeletal system	(i) osteomyelitis and (ii) septic arthirits	(i) Ttypically, an infection of the long bones like femur and humerus, it might also affect the lumbar vertebrae, tibia, radius, and ulna. (ii) the chondrosternal junctions, the hip, the sacroiliac joints, the spine, and the knee	(i) predilection for patients with systemic lupus erythematosus (SLE), diabetes mellitus, lymphoma, liver and cardiovascular diseases, previous surgery or trauma, and patients on steroids. (ii) immunosuppressed individuals or children with congenital diseases where bacteremia has the propensity to spread via hematogenous route to larger joints	*Salmonella* osteomyelitis can be confirmed by simultaneous isolation of *Salmonella* spp. from blood and pus of affected musculoskeletal site, MRI, CT, and radiographs	antimicrobial therapy and surgical debridement	331
skin	Rose spots	red, blanchable macular lesions 2−4 mm in diameter, which show up in 50% of the cases of enteric fever. within the seventh to twelfth day from the onset of symptoms, rose-spots are seen to occur in crops of 5−10 lesions on the lower chest and upper abdomen and may last for 3−4 days		*Salmonella* can be cultured from the puncture biopsies	cured with antibiotics alone without surgical intervention	15

Host Restriction of *S.* Typhi during Macrophage Infection. The inability of *S.* Typhi to thrive inside murine macrophages can be attributed to the absence of virulent gene *gtgE* that codes for a cysteine protease GtgE which is secreted into the host cell cytosol by SPI-2 T3SS.[217,218] Additionally, *S.* Typhi cannot produce the GTPase-activating protein SopD2 due to pseudogenization.[219] While staying in murine macrophages, *S.* Typhimurium employs functionally active GtgE and SopD2 to inhibit the recruitment of Rab29, Rab32, and Rab38 GTPases on the SCV membrane.[219,220] Rab29, Rab32, and Rab38 and their cognate guanine nucleotide exchange factors BLOC1, BLOC2, and BLOC3 are associated with the transportation of melanin-synthesizing enzymes and their phenyl oxidase intermediates with potential antimicrobial activities into lysosome-like organelle called melanosomes.[219,221] In murine macrophage and dendritic cells, the Rab32−BLOC3 pathway helps deliver itaconate, a metabolite that has higher antimicrobial activity and is produced in mitochondria from aconitate decarboxylase, into SCV to restrict the intracellular growth of *S.* Typhi.[222] The pseudogenes in the *S.* Typhi genome facilitate bacterial evolution and enhance their survival fitness inside a human host.

Role of *Salmonella* Pathogenicity Islands of *S.* Typhi in Intramacrophage Survival. Contrary to SPI-1 of *S.* Typhi, SPI-2-encoded T3SS and antimicrobial resistance genes showed markedly increased expression. Unlike nontyphoidal serovars, *S.* Typhi can activate the inflammasome in monocyte-derived macrophages two hours postinfection in a SPI-1-dependent manner.[223] As an early response to *Salmonella* sp. infection in macrophages, the antibacterial oxidative burst is induced by NADPH phagocytic oxidase, followed by the activation of inducible nitric oxide synthase (iNOS), which creates a prolonged bacteriostatic nitrosative burst.[196] Interestingly, macrophage-resident *S.* Typhimurium impairs the NADPH phagocytic oxidase-dependent respiratory burst in an SPI-2-dependent manner.[104,224]

On the contrary, *S.* Typhi infection in human monocyte-derived macrophage cells is independent of SPI-2-encoded translocon protein SseB and basal secretion apparatus SsaR.[225] Using the transposon-directed insertion site sequencing (TraDIS) technique, Karlinsey et al. proved that SPI-2-encoded T3SS of *S.* Typhi is dispensable for the induction of systemic typhoid fever in the humanized mouse model.[226] Expression of the functionally active protein of the *S.* Typhimurium-encoded *marT-fidL* operon in *S.* Typhi (where the same gene is present in the form of a nonfunctional pseudogene) enhanced its susceptibility toward *in vitro* peroxide stress by suppressing the expression of *surV* and aggravated its killing in human monocyte-derived macrophages.[227] The type IVB pili encoded by the *pil* operon located in SPI-7 of *S.* Typhi is responsible for augmenting the expression of NF-KB and proinflammatory cytokine IL-6 in THP-1 cells in a protein kinase C-dependent manner.[228] The inhibition of type IVB pili of *S.* Typhi using a high-affinity single-stranded RNA aptamer ($S\text{-}PS_{8.4}$) impaired bacterial entry in THP-1 cells, implicating an essential role of pili in mediating bacterial interaction with the host macrophage.[229]

Role of Typhoid Toxin in Intramacrophage Survival of *S.* Typhi. The SPI-11 of *S.* Typhi expresses an A_2B_5-type typhoid toxin. It is a multimeric protein complex having catalytically active CdtB (with DNaseI activity) and PltA (with ADP ribosyl transferase activity) domains and five PltB

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ACS Omega 2023, 8, 25674−25697

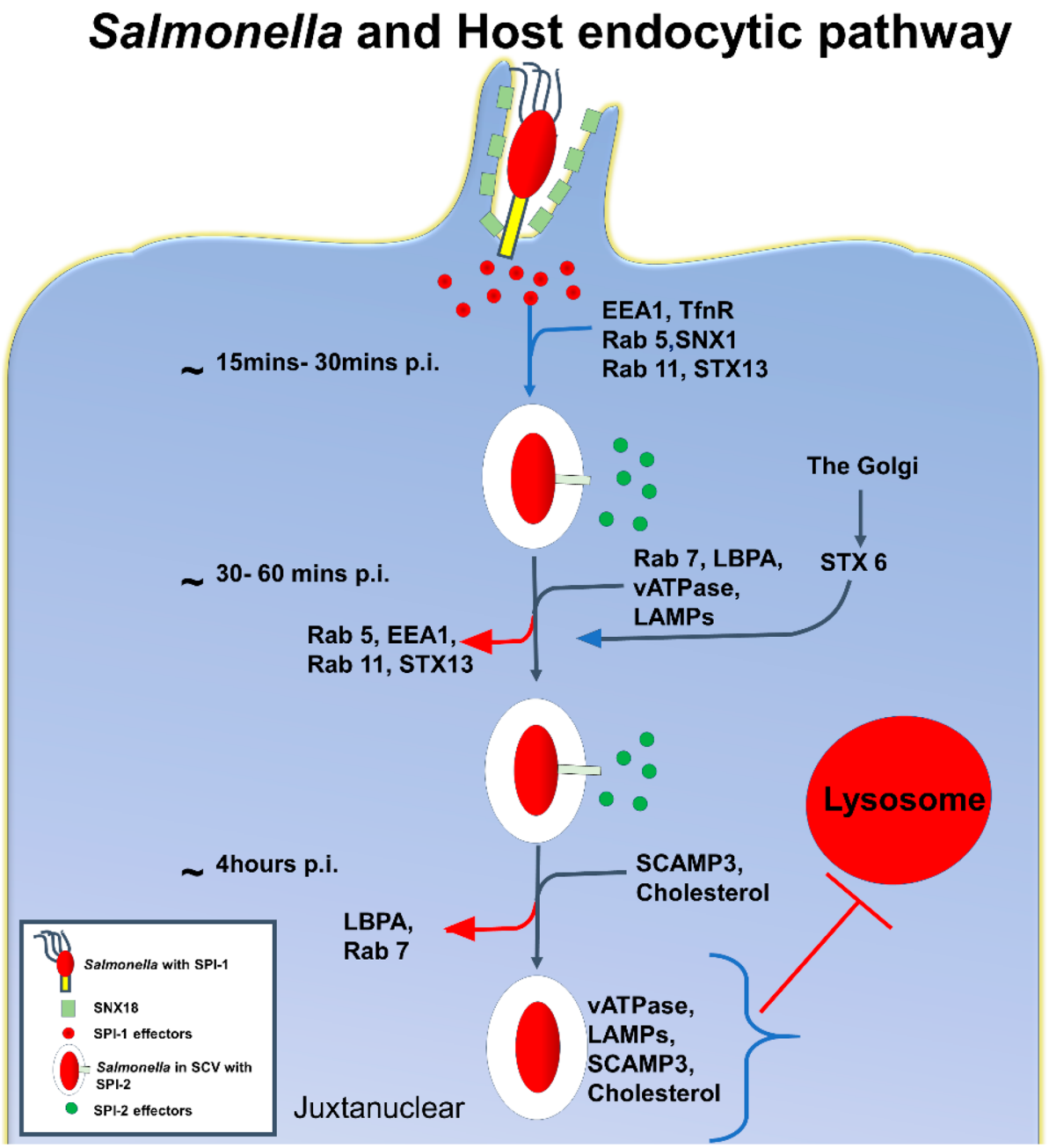

Figure 3. *Salmonella*-containing vacuole (SCV) biogenesis, maturation, and interaction with host endolysosomal pathways.

monomers that constitute the base of the toxin.[123,230] The low Mg^{2+} ion concentration of SCV triggers the PhoPQ two-component system, which activates the *cdtB*, *pltA*, and *pltB* operons encoding the structural and functional subunits of the typhoid toxin and suppresses the expression of histone-like protein H-NS.[231] Deleting the phoP gene from *S.* Typhi attenuated its intracellular survival in epithelial and macrophage cells.[215] Upon secretion of the typhoid toxin from the lumen of SCV, it is transported to the cell membrane with the help of Rab29.[232] After being secreted from the infected cells, it intoxicates the surrounding target cells in an autocrine or paracrine fashion and increases the severity of typhoid fever.[230] The typhoid toxin induces DNA damage in THP-1 cells, eventually resulting in the exhaustion of RPA, a sensor of ssDNA/DNA replication, and the formation of γH2AX foci, which brings senescence. The mechanism behind the replication and senescence has yet to be understood; however, it could be a reason for the higher spread of *S.* Typhi infection.[233] Williams et al. proved that the expression of the CdtB toxin in the nontyphoidal serovar of *Salmonella* sp. *Salmonella* Javiana induces autophagy and cell death in infected macrophages.[234] However, despite the high amino acid sequence similarity between Javiana and Typhoid toxins, there is a difference in the evolutionary host tissue adaptation and cell tropism of both the organisms.[235]

Role of Other Virulent Genes of *S.* Typhi in Intramacrophage Survival. A plethora of virulent genes assist in the survival of *S.* Typhi in macrophages. The global gene expression profile of *S.* Typhi showed that it could also express both SPI-1- and SPI-2-encoded type III secretion systems and virulence factors (SPI-1, *prg*, *sip*, *spa*, and *inv*; SPI-2, *pipB* and *sifB*) during infection and survival within

https://doi.org/10.1021/acsomega.3c02386
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macrophages. In addition, *Salmonella* Typhi can upregulate the expression of several virulence determinants (two-component systems and regulators), such as *phoPQ*, *ssrAB*, and *slyA*, while infecting macrophages.[236] Transposon mutagenesis revealed that mutation in *ssaQ*, *ssaP*, and *ssaN* impaired the intracellular survival of *Salmonella* Typhi in macrophages.[237] In contrast, the uninterrupted intracellular growth of SPI-2-deficient *S.* Typhi showed that neither PhoPQ nor SPI-2 was required for infection in THP-1 cells.[226,225].

The alternative σ-factor *rpoS* that regulates the expression of *spv* (*Salmonella* plasmid virulence) during the systemic infection of *S.* Typhimurium plays a vital role in the virulence of *S.* Typhi. Deleting *rpoS* from the *S.* Typhi genome makes the bacteria susceptible to NO-dependent host defense even inside both murine (RAW264.7) macrophages.[238] Wang et al. reported that deleting *sufC* enhances *Salmonella* Typhi's susceptibility toward oxidative stress and reduces its survival inside the macrophages.[239] The virulence-associated capsular polysaccharide (Vi antigen) of *S.* Typhi plays a diverse role in its survival inside phagocytes. It can abolish the nitric oxide- and reactive oxygen species-dependent innate immune response in monocyte-derived macrophage cells and neutrophils.[240] In *S.* Typhi, the expression of the Vi capsular antigen is positively regulated by OxyR, which eventually induces hydrogen peroxide inducible genes.[241,242] Further, the Vi capsular antigen results in reduced binding of complement component 3 (C3) on the surface of *S.* Typhi, thus rendering protection against complement-mediated phagocytosis and preventing its clearance from the liver, spleen, and blood of mice.[243] The Vi antigen-dependent abrogation of IL-8 expression in infected THP-1 cells is the reason behind the deficiency of neutrophils in intestinal infiltrates of patients suffering from typhoid fever.[244] Due to the presence of the Vi capsular antigen around its surface, *S.* Typhi can restrict the recruitment of complement component 5a (C5a) and thus inhibit the C5aR-dependent chemotactic movement of neutrophils.[245] *S.* Typhi fimbriae also regulate phagocytosis and intracellular survival in THP-1 cells.[246] *S.* Paratyphi A uses the O2 antigen to prevent the binding of immunoglobulin IgM and thus inhibit the respiratory burst in primary murine and human neutrophils.[247]

Karlinsey and colleagues showed the importance of iron acquisition and utilization during *in vivo* infection caused by *S.* Typhi, where the *iroCDEN* mutant could not induce typhoid fever in hu-SRC-SCID mice.[226] While infecting THP-1 macrophages, wild-type *S.* Typhi secretes eukaryote-like serine-threonine kinase from SCV into the host cytoplasm, which phosphorylates myelin basic proteins (MBPs) and eventually enhances the expression of proinflammatory cytokines like TNF-α and IL-6.[248] The bacterial noncoding RNA (ncRNAs) *malS* are crucial in regulating *S.* Typhi virulence inside the macrophage. The intramacrophage life of *S.* Typhi was notably hampered by the overexpression of 5′ UTR of *malS* (*malS*-5′UTR), which downregulated the expression of *mgtC* that maintains ATP homeostasis in the bacteria.[249] A study conducted by He et al. implicated that pR_{ST98}, a hybrid resistance−virulence plasmid of *S.* Typhi, is responsible for suppressing autophagy and promoting the survival of the bacteria in infected macrophages.[250] Recent studies demonstrated that nontyphoidal *Salmonella* serovars widely use numerous novel virulence factors, such as outer membrane proteins (OmpA and PgtE), YdcP, and a novel putative collagenase, to interact with host macrophages.[251−254] High sequential homology for these novel virulence factors between *Salmonella* Typhi and Typhimurium indicated their involvement during typhoid fever. However, thorough and detailed research is required to annotate their exact functions in *S.* Typhi.

S. Typhi Infection in Dendritic Cells. During chronic *Salmonella* infection, the greater abundance of macrophages, neutrophils, and dendritic cells in the spleen indicated that *S.* Typhi could reside in dendritic cells (DCs), neutrophils, and B lymphocytes.[255] Dendritic cells can capture bacteria from the lumen of the intestine. With the help of cell surface MHC II molecules and other costimulatory molecules like CD40 receptors, CD80, and CD86 ligands, DCs present bacterial antigens to the $CD4^+$ helper T lymphocyte population and induce an adaptive immune response. While infecting murine DCs, *S.* Typhimurium delays the recruitment of NOX2 on SCV and impairs antigen presentation by K63-linked ubiquitination of MHC-II and subsequent degradation by endosomal proteases.[256] *S.* Typhimurium infection in murine DCs enhances the expression and activity of iNOS (NOS2), which produces RNS to limit the growth of the bacteria in the SIRT2-dependent manner.[257] During *S.* Typhi infection, intestinal inflammation is dictated by the pathogen and gut microbiota's interactions with intestinal DCs. The coinfection of intestinal DCs with a probiotic strain of *Bifidobacterium breve* CNCM I-4034 and human pathogen *S.* Typhi enhanced the expression of proinflammatory cytokines and chemokines like TNF-α, IL-8, and RANTES in a TLR-2-dependent manner.[258] Infection of bone marrow-derived dendritic cells (BMDCs) with *S.* Typhi bacterial ghost (STG, which is an inactivated vaccine candidate expressing the *lysisE* gene from DNA phage ϕX174), triggered the maturation of $CD11c^+$ BMDCs by increasing the expression of CD40, CD80, and MHC-II molecules.[259] Furthermore, in DCs, the viable *S.* Typhi deploys several virulence factors to manipulate the host signaling process to favor its growth. The pR_{ST98} of *S.* Typhi suppresses the surface expression of several costimulatory molecules, such as CD40, CD80, and CD86, inhibiting autophagy in DCs. Wild-type *S.* Typhi can escape the Th1 response by downregulating IL-12 and IFN-γ and upregulating IL-10.[260] In line with this observation, BALB/c mice infected with *S.* Typhimurium SR11 x3337 bearing the pR_{ST98} plasmid of *S.* Typhi showed suppression of dendritic and T cell activation.[261] On the contrary, human dendritic cells cocultured with the PBMCs infected with *S.* Typhi produced a higher level of proinflammatory cytokines like 12p70 and IFN-γ and augmented the antigen presentation to primed $CD3^+$ and $CD8^+$ effector/memory T cells.[262] *S.* Typhi utilizes TviA, a regulatory protein associated with flagellin expression (*fliC*), to invade the intestinal mucosa and escape antigen presentation by dendritic cells. The expression of *S.* Typhi TviA in *S.* Typhimurium diminished antigen presentation by dendritic cells to $CD4^+$ T lymphocytes by lowering the availability of flagellar antigen in the $Nramp^{+/+}$ C57BL/6 mouse.[263] However, the complex behavior of *S.* Typhi in dendritic cells has been poorly understood, and a considerable amount of research is required to unveil the mechanisms employed by the pathogen to modulate DCs and to thrive inside an otherwise hostile niche. Table 3 summarizes the strategies that *Salmonella* uses to emerge as a successful pathogen.

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ACS Omega 2023, 8, 25674−25697

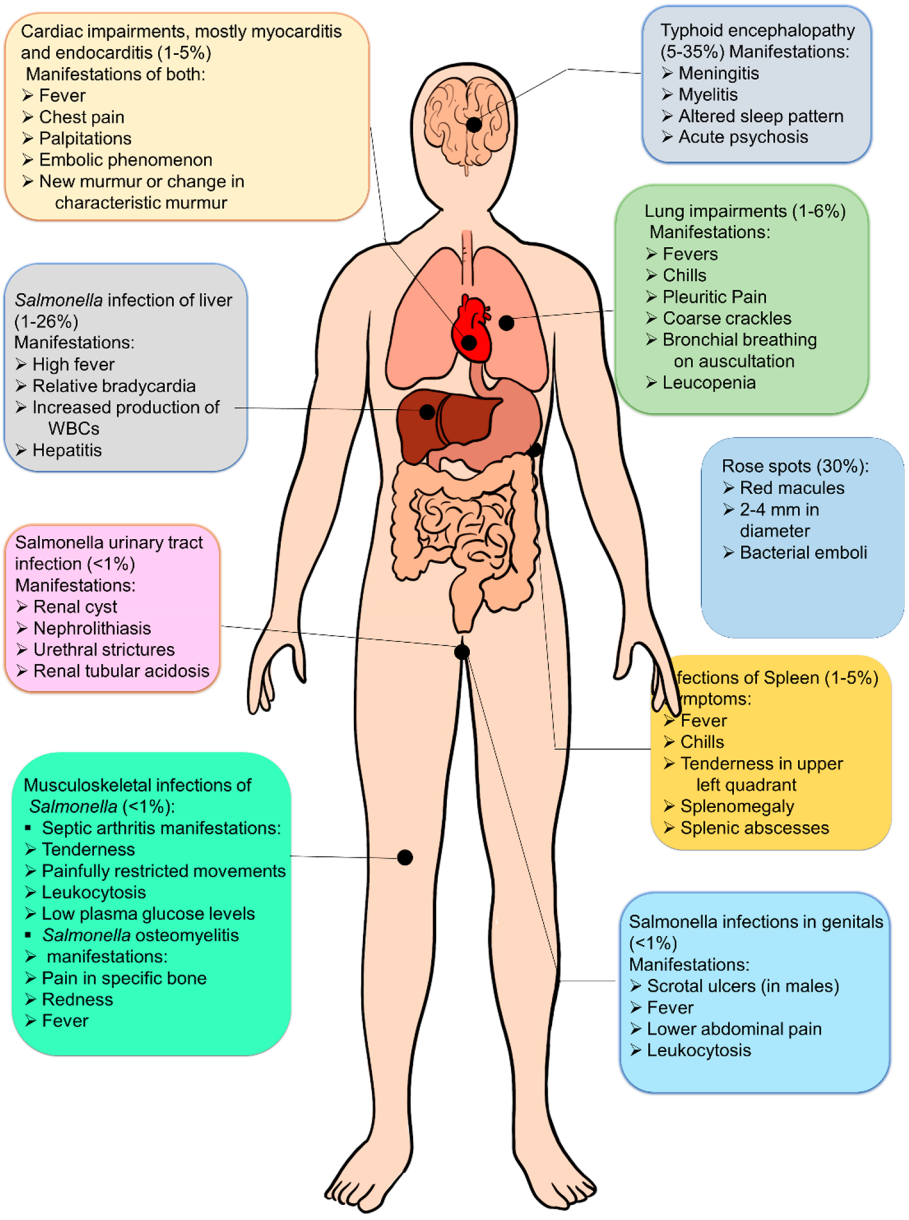

Figure 4. Extra-intestinal infections caused by *Salmonella* Typhi. The spleen is located behind the stomach, and kidneys are located at the back of the trunk's middle and have been marked accordingly. The percentage of occurrence is expressed concerning total gastrointestinal infections caused by the pathogen.

EXTRA-INTESTINAL INFECTIONS CAUSED BY *S. TYPHI*

Salmonella multiplies in reticuloendothelial cells and macrophages of the liver, spleen, lymph nodes, and bone marrow during the asymptomatic phase of infection. If left untreated, bacteria reach a threshold level and are released in the blood, initiating secondary bacteremia with cytokine secretion during the symptomatic phase of typhoid fever.[264] This secondary persistent bacteremia phase leads to the seeding of other organs, which may further complicate the situation by developing intestinal perforations, hepatitis, pneumonia, and tissue abscesses, usually during the second and fourth weeks of illness.[40,41,265,266]

Hence, it will be appropriate to comment that once bacteremia sets in following an *S.* Typhi infection, almost all organs have the potential to host bacterial foci. Quite frequently diagnosis of this infection is ignored, which leads to fatal outcomes. Imaging techniques such as magnetic resonance imaging (MRI), ultrasonography (USG), and radiographs have facilitated the diagnostic process. MRI is a medical imaging technique used in radiology, and it can visualize the anatomy and physiological processes of the body. USG can help diagnose systemic manifestations like enlarged mesenteric lymph nodes (MLNs), mural thickening of terminal patients, splenomegaly, and acute acalculous cholecystitis associated with *Salmonella* infection. However, culturing the pathogen from the site of infection is the ultimate gold-standard diagnostic tool. Figure 4 and Table 4 demonstrate summarys of the extraintestinal infections of *Salmonella* and their manifestations.

CONCLUSION

Salmonella enterica infection remains a significant public health concern worldwide, especially in third-world countries where

https://doi.org/10.1021/acsomega.3c02386
ACS Omega 2023, 8, 25674−25697

Salmonella infection is often endemic due to various socio-economic factors. The history of epidemics, genetic makeup, and stealthy strategies against the host makes *Salmonella* a superbug. Its adaptation to infect a range of hosts often makes eradicating this notorious pathogen several times difficult. Despite significant progress in medical science over the last centuries, *Salmonella* causes significant mortality and morbidity pertaining mainly to its adaptation to host defense and the evolution of drug-resistant strains. *Salmonella* can cleverly manipulate host defense arsenals, thereby mounting an intense infection in the host system. This Review discussed the essential aspects that help the bacteria achieve this, especially horizontally acquired islands (SPIs). Therefore, understanding the biology of *Salmonella* infection is essential and will help us to reduce the burden of *Salmonella* infection globally.

Although typhoidal and nontyphoidal *Salmonella* serovars belong to the same biological species, these two serovars mount and trigger distinct acute infections in the host. The situation worsens due to extraintestinal manifestations, carriage, and recurrent infections in the population. The field of *Salmonella* biology has an immense amount of literature on understanding the molecular mechanisms of virulence. Nevertheless, there is a dearth of accurate information regarding the *in vivo* scenario due to host restrictions and the unavailability of permissive animal models. Therefore, there is a pressing need for a different animal model, a collaborative system of biology insights, and advanced techniques to decipher the systemic effects of *Salmonella* infection and single-cell approaches. This will further help us to develop plausible novel therapeutics and vaccination strategies against *Salmonella* infections.

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ACKNOWLEDGMENTS

We are thankful for the financial support from the Department of Biotechnology (DBT) and Department of Science and Technology (DST), Ministry of Science and Technology. D.C. acknowledges DAE for the SRC outstanding investigator award and funds, a TATA fellowship, and ASTRA Chair Professorship funds. The authors jointly acknowledge the DBT-IISc Partnership Programme. Infrastructure support from ICMR (Center for Advanced Study in Molecular Medicine), DST (FIST), and UGC-CAS (special assistance) is acknowledged. R.C. duly acknowledges the CSIR-SRF fellowship. A.R.C. acknowledges Shamrao M. Kaikini and Krishna S. Kaikini's fellowship. D.M. acknowledges the IISc fellowship.

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