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One species, different diseases: the unique molecular mechanisms that underlie the pathogenesis of typhoidal *Salmonella* infections

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Abstract

Salmonella enterica is one of the most widespread bacterial pathogens found worldwide, resulting in approximately 100 million infections and over 200,000 deaths per year. *Salmonella* isolates, termed "serovars", can largely be classified as either non-typhoidal or typhoidal *Salmonella*, which differ in regard to disease manifestation and host tropism. Non-typhoidal *Salmonella* causes gastroenteritis in many hosts, while typhoidal *Salmonella* is human-restricted and causes typhoid fever, a systemic disease with a mortality rate of up to 30% without treatment. There has been considerable interest in understanding how different *Salmonella* serovars cause different diseases, but the molecular details that underlie these infections have not yet been fully characterized, especially in the case of typhoidal *Salmonella*. In this review, we highlight the current state of research into understanding the pathogenesis of both non-typhoidal and typhoidal *Salmonella*, with a specific interest in serovar-specific traits that allow human-adapted strains of *Salmonella* to cause enteric fever. Overall, a more detailed molecular understanding of how different *Salmonella* isolates infect humans will provide critical insights into how we can eradicate these dangerous enteric pathogens.

Keywords

enteric fever; diarrhea; salmonella; Evolution; virulence; pseudogenes; pathogenesis; persistence; granulomas; macrophages; macrophage polarization

1. Introduction to typhoidal *Salmonella*

Salmonella enterica subspecies *enterica* is comprised of over 2,400 serovars that infect animal hosts via the fecal-oral route, resulting in a range of disease states ranging from self-limiting gastroenteritis to lethal systemic infection [1]. Typhoidal *Salmonella* serovars S.

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Typhi and S. Paratyphi A are clinically important, causing over 200,000 deaths world-wide and a significant burden on developing, under-resourced healthcare systems [2]. The H58 lineage of *S.* Typhi is particularly problematic, as it is strongly associated with antimicrobial resistance and is rapidly becoming the most prevalent typhoidal lineage across the world [3]. Most individuals infected by typhoid fever live in South and Southeast Asia [4], and the emergence of an extensively drug-resistant (XDR) strain in these densely populated areas is extremely worrisome [5]. Infections with Typhi and Paratyphi A, which are human-restricted pathogens, usually begin with ingestion of contaminated water or food. The pathogens invade the gastrointestinal mucosa, translocate to the lymphoid follicles where they survive and replicate within macrophages, and then disseminate to the intestinal lymph nodes, liver, spleen, bone marrow and gallbladder [4]. Vaccinations against typhoid fever are available but differ in regard to the protection they offer: the live oral Ty21a vaccines elicits some level of cross-protection against multiple strains of *Salmonella* [6], while the typhoid conjugate vaccines do not provide protection against paratyphoid fever, a cause of one in five cases of enteric fever in some areas of Asia [7]. Thus, an increase in paratyphoid fever cases in endemic areas is predicted to occur [8].

The restriction of Typhi and Paratyphi A to human hosts limits experimental approaches available to study the pathogenesis of enteric fever caused by these serovars. Typhimurium infection in mice is commonly considered a model system for studying the pathogenesis of typhoid and paratyphoid fever, but important differences among these serovars exist. For example, while both non-typhoidal and typhoidal *Salmonella* encode two type 3 secretion systems (T3SSs) on *Salmonella* pathogenicity islands (SPI) 1 and 2, which are required for translocating effector proteins into mammalian cells, prior studies have shown that the SPI-2 T3SS is not required for the intracellular survival and replication of typhoidal *Salmonella* in the human macrophage (MΦ) cell line THP-1 [9,10] or for infection in a humanized mouse model [11]. In addition, many T3SS effector genes are either absent or pseudogenes in Typhi and Paratyphi A, and thus the functions of T3SS-1 and −2 and their effectors may be absent or altered in Typhi and Paratyphi A [1]. In this review we will discuss the genetic basis for divergent pathogenicity between serovars, key bacterial virulence factors underlying typhoid and paratyphoid infection, and the human immune basis for heterogeneous clinical outcomes (Fig. 1).

2. Evolution of human-restricted *Salmonella* serovars: divergence of typhoidal serovars from generalist serovars

The genetic basis underlying host-restriction of divergent *Salmonella enterica* serovars is a topic of considerable interest. While typhoidal and non-typhoidal *Salmonella* share >90% of genes, these serovars also encode hundreds of unique genes that may underpin differences in disease manifestations [12]. Many serovar-specific genes are within integrated prophage regions, which represent important sites of genetic diversity across bacterial genomes (Table 1). Prophages often encode “cargo” genes that can significantly impact microbial behavior and virulence. For example, multiple genes encoding type III secretion system (T3SS) effectors are contained on prophage regions across *Salmonella* serovars, including *sopE, sspH1, sseI*, and *sopE2*. The importance of T3SSs during typhoidal *Salmonella* virulence

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is discussed in more detail in Section 3 of this review. Other serovar-specific genes are found on *Salmonella* Pathogenicity Islands (SPIs), which are large, horizontally acquired genetic cassettes that often encode virulence genes (Table 1). For example, SPI-7,−15,−17 and −18 are unique to typhoidal serovars, while SPI-14 is only found in non-typhoidal isolates. SPI-7 is an especially important region of the *S.* Typhi genome that encodes the Vi antigen, a capsular polysaccharide that coats the surface of the bacterium and contributes to virulence. More specifically, the Vi capsule prevents complement binding [13] and masks lipopolysaccharide (LPS) from TLR-4 recognition [14], thereby avoiding immune recognition. The Vi capsule has also recently been found to promote phagocytosis of *S.* Typhi by human macrophages while simultaneously allowing *S.* Typhi to evade phagocytosis by human neutrophils [15]. TviA, the transcriptional activator of Vi production, is contained within SPI-7 and can also suppress the expression of flagellin and T3SS-1, thereby allowing *S.* Typhi to evade TLR-5 and NAIP-mediated inflammatory responses [16]. Together, these findings suggest that Vi capsule production contributes to a “stealth” *S.* Typhi phenotype, which may allow *S.* Typhi to disseminate to systemic sites without eliciting strong inflammatory immune responses. Interestingly, *S.* Paratyphi A also causes enteric fever but does not encode the Vi capsule. Instead, *S.* Paratyphi A encodes *fepE*, which leads to the generation of very long O-antigen chains that help this pathogen escape the immune response by limiting inflammasome activation and subsequent cell death [17]. While non-typhoidal strains of *Salmonella* also encode *fepE*, previous studies have shown that *S.* Paratyphi A expresses *fepE* at significantly higher levels compared to *S.* Typhimurium [17]. Modification of surface antigens may therefore be a convergently evolved mechanism of Typhi and Paratyphi A to evade the human immune system.

Several pathogenicity islands are also shared between typhoidal and non-typhoidal *Salmonella*, but there are many serovar-specific genes encoded within these regions. For example, SPI-11 is present in both non-typhoidal and typhoidal serovars, but this island contains genes that encode the typhoid toxin in *S.* Typhi and *S.* Paratyphi A, including *cdtB* and *pltAB* [18]. While the molecular details of how typhoid toxin benefits *Salmonella* in the host are still being investigated, previous work has shown that the typhoid toxin causes DNA damage and host cell cycle arrest in cultured intestinal epithelial cells [19] and in human primary gallbladder cells [20]. There are also observations linking the toxin to gallbladder cancer through a mechanism where the CdtB subunit causes DNA damage and subsequent activation of the p53/P21 axis [21], and indeed previous work has shown that patients who are carriers of typhoidal *Salmonella* are significantly more likely to develop carcinomas in the gallbladder compared to non-carriers [22]. In addition, researchers have shown that introducing typhoid toxin-encoding genes to *S.* Typhimurium increases the frequency at which asymptomatic carriers emerge in long-term murine models of *Salmonella* infection [23], while another study assessing a two-day infection of *S.* Typhi in humanized mice did not observe a typhoid toxin-dependent effect [11]. In agreement with this mouse study, one recent study in humans has suggested that the typhoid toxin is not required for virulence at the acute stage of typhoid fever [24]. Together, these studies suggest that this toxin may play a role in systemic virulence and long-term persistent infection, but may not be necessary during early stages of typhoid fever.

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Another distinguishing feature of typhoidal serovars is the large proportion of pseudogenes that are present in their genomes, which is a hallmark of genome degradation. Approximately 5% of the genome in sequenced typhoidal isolates are predicted to be pseudogenes, which arise either from single base mutations that result in frameshift mutations, or from the appearance of early stop codons [12]. These pseudogenes include loci that encode many different functions, including metabolic genes (ie, *narW, wcaK*), transporters (i.e., *fhuA, mglA*), chemotaxis genes (i.e., *trg*), and cell-wall related genes (i.e., *dacD*) [25,26]. One study has shown that the loss of the loss of the *ydiQRSTD* operon in *S.* Typhi, which is responsible for the utilization of butyrate by non-typhoidal *Salmonella*, may have contributed to the transition of *S.* Typhi from a gastrointestinal to an extraintestinal pathogen [27]. Interestingly, there are multiple virulence genes in *S.* Typhimurium that are pseudogenes in *S.* Typhi and/or *S.* Paratyphi A, including several SPI-1 and SPI-2 T3SS effectors (i.e., *sopA, sseI, steB, sopE2, sseK2*, and *sopD2*) [25]. *sseI* is also a pseudogene in non-typhoidal *Salmonella* belonging to the ST313 lineage, which causes systemic bacteremia in humans [28]; these invasive non-typhoidal *Salmonella* infections have been reviewed elsewhere extensively [29-32]. Previous work has shown that the loss of SseI contributes to the ability of ST313 *Salmonella* to hyperdisseminate from the gut to systemic sites [33] and thus the pseudogenization of *sseI* may contribute to the ability of ST313 *Salmonella* to cause systemic bacteremia. In addition, many genes encoded by non-typhoidal *Salmonella* that contribute to colonization of the gut are pseudogenes in *S.* Typhi and *S.* Paratyphi A, including the autotransporter *misL*, the adhesin-encoding gene *siiE*, and the type VI secretion system (T6SS) genes *sciI* and *sciS* [12]. It is likely that the loci mutated in *S.* Typhi and *S.* Paratyphi A are not necessary for systemic disease in humans. In agreement with this hypothesis, many of these virulence-related pseudogenes have been shown in *S.* Typhimurium to promote intestinal persistence, which is not a hallmark of typhoidal serovars [12]. More generally, gene loss has been found to result in fundamental changes in the behavior of numerous bacterial pathogens and can even enhance virulence phenotypes [34-38]. In the case of *S.* Typhi, it has been reported that the loss of the *fepE* gene enhances immune evasion mediated by the Vi capsule [39].

Although both *S.* Typhi and *S.* Paratyphi A encode many pseudogenes, there is little overlap in pseudogenes between these typhoidal serovars; for example, less than 20% of pseudogenes in *S.* Paratyphi A 9150 A are also pseudogenes in *S.* Typhi CT18 [25]. This observation strongly suggests that *S.* Typhi and *S.* Paratyphi A have evolved independently to converge on the same disease phenotype. Based on the high number of pseudogenes in these serovars, it is likely that both *S.* Typhi and *S.* Paratyphi A primarily evolved via reductive evolution from a non-typhoidal ancestor, losing genes that are no longer necessary for survival or intracellular replication during the progression of typhoid fever [40]. Interestingly, reductive evolution appears to be a feature of other human-restricted pathogens including *Mycobacterium leprae, Shigella flexneri*, and human-restricted strains of *Bordetella spp* [41-43]. In addition, recent work has suggested that some bioinformatically predicted pseudogenes in typhoidal *Salmonella* may produce functional proteins, although this finding should be confirmed experimentally by other groups [44]. Thus, a more careful analysis of pseudogenes in the above pathogens may yield deeper

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insights into the molecular mechanisms by which human-restricted pathogens evolve to cause disease.

3. Molecular mechanisms underlying typhoid and paratyphoid pathogenesis

As previously mentioned, *Salmonella* encodes two T3SS, which are conserved between non-typhoidal and typhoidal *Salmonella* [45]. However, the repertoire of T3SS effectors differ between these serovars (Table 2). The roles that T3SS-secreted effectors play are best described for Typhimurium infections – ranging from manipulation of host cytoskeleton to immune evasion, intracellular trafficking and cell survival [45-47]. Differences in expression of T3SS and the effector repertoire between typhoidal and non-typhoidal serovars will be highlighted here.

T3SS-1, encoded in the SPI-1 locus, enables *Salmonella* species to gain a foothold in the gastrointestinal tract by triggering *Salmonella* uptake by non-phagocytic cells, including epithelial cells. Previous work has shown that the regulation of T3SS-1 dependent invasion may differ between non-typhoidal and typhoidal *Salmonella*. For example, Winter et. al. found that TviA, a transcriptional regulator that is only present in *S.* Typhi, positively regulates Vi capsule production and represses T3SS-1 expression [48]. In addition, *S.* Typhi up-regulates T3SS-1 and invasion of epithelial cells in response to bile, which is present in the gallbladder, whereas *S.* Typhimurium does not [49], which may partly explain why the gallbladder is a site of chronic *S.* Typhi infections in humans [50].

In addition to differences in T3SS regulation, the effectors encoded on these virulence loci also differ between non-typhoidal and typhoidal *Salmonella* (Table 2). For example, previous work has shown that many T3SS-1 effectors contribute to intestinal inflammation in *S.* Typhimurium [51], but some of these effectors are pseudogenes in *S.* Typhi and *S.* Paratyphi A. In turn, one hypothesis for why typhoidal serovars do not cause acute intestinal inflammation is genetic degradation of the specific T3SS-1 dependent effectors. For example, six T3SS-1 effectors, SipA, SopA, SopB, SopD, SopE, and SopE2, elicit neutrophil infiltration and fluid accumulation during *S.* Typhimurium infection of bovine illeal loops [52], but SopA and SopE2 are pseudogenized in *S.* Typhi [53]. Another potential hypothesis for reduced intestinal inflammation is that typhoidal serovars encode unique genes to specifically dampen inflammatory responses during intestinal infection. Interestingly, a T3SS-1 dependent effector present in *S.* Typhi, named StoD, is an E3/4 ubiquitin ligase that likely causes degradation of host targets and may play a role in dampening immune responses [54].

After adhering to the surface of epithelial cells, T3SS-1 injects effector proteins into the host cell cytoplasm to facilitate internalization. *S.* Typhi and *S.* Typhimurium both induce T3SS-1 dependent ruffling of epithelial cell surfaces upon invasion, indicating a conserved “trigger” mechanism of invasion caused by cytoskeletal rearrangement. The injected effectors SipA and SipC directly nucleate actin [55], while SopE and SopE2 initiate actin polymerization through host GTPases Cdc42 and Rac1[56]. Notably, SipA, SipC and SopE are conserved in typhoidal serovars while SopE2 is pseudogenized [53]. The T3SS-1 dependent effector SptP

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is deployed by *S.* Typhimurium to antagonize SopE-mediated cytoskeletal rearrangements by degrading Cdc42 & Rac1 and return the cytoskeleton to homeostasis after internalization occurs [57]. However, the *sptP* ORF within *S.* Typhi contains a point mutation rendering the protein non-functional [58]. Therefore, the mechanism by which *S.* Typhi resolves host cytoskeletal rearrangements post-invasion is currently unclear.

While T3SS-1 dependent invasion is required to enter nonphagocytic cells, macrophages naturally phagocytose pathogens, and thus SPI-1 mediated invasion is not required for *Salmonella* internalization [59]. Paradoxically, macrophages, which typically kill phagocytosed pathogens, are a major replicative niche for *Salmonella* during systemic infection [60]. *Salmonella* uses multiple molecular mechanisms to facilitate intra-macrophage replication. Effector proteins deployed through the T3SS-2 are required for *S.* Typhimurium intra-macrophage replication within the *Salmonella*-containing vacuole (SCV) [61]. However, T3SS-2 is not strictly required for *S.* Typhi intracellular survival and/or replication in THP-1 macrophages [9,10]. The molecular mechanisms underlying this discrepancy between non-typhoidal and typhoidal serovars remain unclear.

Most of the mechanisms by which *Salmonella* survive and replicate within a vacuole inside of macrophages has been performed with S. Typhimurium [62]. For example, previous studies have shown that two T3SS-2 dependent effectors, SseF and SseG, play an important role in intracellular trafficking of the SCV [63], and these effectors are conserved in typhoidal *Salmonella*. It has also been shown in *S.* Typhimurium that multiple effectors, including SifA, PipB2, SopD2 and SseJ, contribute to growth and elongation of the SCV membrane to form *Salmonella*-induced filaments (Sifs) [64]. However, these Sif-inducing effectors are not universally conserved; for example, SseJ, which normally localizes to the SCV surface and recruits SifA in Typhimurium, is a pseudogene in typhoidal *Salmonella* [65]. Similarly, the gene that encodes SopD2, which plays a role in limiting Sif extension and ensuring optimal membrane dynamics, is also a pseudogene in typhoidal *Salmonella* [26]. Whether *S.* Typhi and *S.* Paratyphi A genomes contain different effectors that function similarly to SseJ and SopD2 is currently unclear. In *S.* Typhimurium, SopD2 also works cooperatively with another effector protein, GtgE, which is absent in typhoidal serovars [66]. GtgE degrades various Rab GTPases, which typically regulate many components of membrane trafficking in eukaryotic cells and may deliver antimicrobial factors to the SCV during *Salmonella* infection [67]. Previous work has shown that the presence of Rab32 leads to the death of *S.* Typhi in mouse macrophages, which may partly explain why typhoidal *Salmonella* cannot infect mice [66]. In agreement with this model, trans-expression of GtgE from *S.* Typhimurium allows *S.* Typhi to overcome host restriction by removing Rab32 from the SCV surface and replicate in murine macrophages [66]. While typhoidal *Salmonella* does not encode GtgE, one recent study has suggested that *S.* Typhi utilizes its SPI-1 T3SS to evade Rab32-mediated killing in human macrophages [68]. The molecular mechanisms by which *S.* Typhi can overcome the same Rab-mediated killing in human macrophages but not in murine macrophages are currently unclear.

While both typhoidal and non-typhoidal *Salmonella* survive and thrive within macrophages, the fate of intracellular *Salmonella* in human macrophages are heterogeneous, and include host-killed, non-replicating, persisting, and actively replicating intracellular bacteria [69]. As

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demonstrated with *S.* Typhimurium, macrophages with host-killed bacteria and bystander cells share a transcriptomic phenotype that is characterized by an M1 polarization state [70]. In contrast, macrophages that allow for intracellular replication and non-replicating persisting bacteria tend to have a transcriptomic profile dominated by M2 polarization genes [70]. Thus, the state of individual macrophages influences the ability of intracellular *Salmonella* to replicate and manipulating macrophage activation states can alter the overall outcome of *Salmonella* infection. Whether or not these bacterial populations and/or macrophage polarization states are different during typhoidal vs. non-typhoidal *Salmonella* infections is currently an ongoing area of investigation. Determining the relative sizes of each of these populations, their role in maintaining persistent infection, and characterizing the molecular mechanisms underlying each state may reveal additional serovar-specific differences during macrophage infection.

4. Host factors that influence the outcome of disease

While multiple differences have been identified between non-typhoidal and typhoidal *Salmonella* that may influence disease manifestation, as highlighted in the above sections, there are also numerous studies investigating differences in host factors and genetic predispositions that may influence disease severity in the infected host. A variety of pathogen-associated molecular patterns (PAMPs) in both typhoidal and non-typhoidal *Salmonella* interact with extracellular and intracellular pattern recognition receptors (PRRs). For example, TLRs including TLR2, TLR4 and TLR5 recognizes CsgA, LPS and FliC, respectively [71-73]. As these receptors are engaged, the adaptor proteins TIRAP/MyD88 or TRIF/TRAM are phosphorylated and initiate distinct signaling cascades that lead to cytokine and chemokine production, which recruit and activate myeloid and lymphoid cells necessary to clear the infection [74]. Consequently, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$ and *Myd88*$^{-/-}$ mice have distinct disease phenotypes and succumb to infection within days of *S.* Typhimurium infection [75]. TLRs are highly involved in inducing inflammasome formation and the subsequent processing and release of IL-1 superfamily proteins which is the hallmark of pyroptosis [76]. As such, inflammasome deficient mice, including *Casp1*$^{-/-}$, *Il18*$^{-/-}$ and *Il1b*$^{-/-}$ are more susceptible to *S.* Typhimurium infection than wild type control mice [77]. In addition, one study has shown that TLR11 is responsible for host restriction of *S.* Typhi in mice [78], although other groups have failed to replicate this finding [79].

Other factors beyond PRRs have also been shown to influence host susceptibility to *Salmonella*. For example, a point mutation in the *Nramp1* allele, which regulates iron-availability in macrophages, has also been shown to increase the susceptibility of conventional C57BL/6J and BALB/C mice to *Salmonella* infection, and C57BL/6 mice complemented with the wild type *Nramp1* allele have increased survival compared to WT controls [80]. In addition, MHC-II deficient mice that fail to present *Salmonella* antigens to host immune cells are more susceptible to infection than wild type mice [81]. Some of these afore-mentioned genes have further been implicated to play a role in the susceptibility to typhoid fever in humans, suggesting that there are shared immunological pathways that are important for causing more severe disease in both humans and animals. For example, SNPs in both *TLR4* [82] and *HLA* genes [83] has been shown to influence typhoid susceptibility. In contrast, there are some genes that may influence infection outcomes

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differently in different hosts. For example, while *Nramp1* is important for control of non-typhoidal *Salmonella* infections in mice [80,84], there are no reports that SNPs in the human homologue *SLC11A1* influence typhoid susceptibility [85]. In addition, SNPs in *IL4* [86], *PARK2* [87], *PACRG* [87], *CFTR* [88] *and VAC14* [89] have been implicated to increase the susceptibility to typhoidal *Salmonella* infection in humans, but they have not been shown to have effects in murine models. Studies have also shown that defects in the IFNγ-IL12 axis are risk factors for invasive non-typhoidal *Salmonella* infections, but not for typhoid fever [90-92].

While mouse models are commonly used to study non-typhoidal *Salmonella* infections in the laboratory, there are critical differences between human and murine cells that are important to consider. For example, *Salmonella* infection activates the NAIP/NLRC4 inflammasome in both human and murine macrophages, but these responses are distinctly different. Mice encode multiple NAIPs which are constitutively expressed by macrophages to sense bacterial ligands in the cytosol while humans encode a single NAIP [93]. The NLRC4/NAIP inflammasome recognizes SPI-1 and SPI-2 associated proteins (PrgI, PrgJ and SsaG) as well as flagellin [94]. For human NAIP to recognize flagellin, a full-length isoform is needed which is expressed in hMDMs but not in monocytic/macrophage cell lines such as THP-1 or U937 cells [95]. On the pathogen side, *Salmonella* serovars have evolved different mechanisms to counteract host recognition and inflammasome activation. For example, non-typhoidal strains of *Salmonella* within the ST313 lineage trigger less inflammasome activation compared to serovars belonging to the ST19 lineage [28]. Differences in these responses have been attributed to lower expression of the SPI-1 effector SopE2 and the flagellin FliC in ST313 strains compared to ST19 isolates during host cell infection [28]. Typhoidal *Salmonella* also triggers lower amounts of inflammasome activation compared to non-typhoidal *Salmonella*, in part due to the presence of the TviA transcriptional regulator, which is uniquely found in typhoidal *Salmonella*. TviA decreases the expression of flagellin during host cell infections by *S.* Typhi, in turn suppressing inflammasome activation and subsequent IL-1β and pyroptosis responses [16].

One hallmark of typhoid fever is that ~5-10% of people carry *Salmonella* persistently for more than 1 year and thus serve as a reservoir of infection for this disease [96]. Although our understanding of the mechanisms that underlie typhoidal persistence remains limited, it has been shown that typhoidal *Salmonella* can persist in the gallbladders of chronic carriers [97]. Intriguingly, bile, which is found at high concentrations in the gallbladder, has been shown to trigger increased biofilm formation in typhoidal *Salmonella* [98], which may partly explain how this pathogen is able to persist in the gallbladder. It has also been reported that not all chronic carriers of typhoidal *Salmonella* have gallstones [99], strongly suggesting that there are other factors that contribute to the persistent stage of this infection. However, given the human-restricted nature of typhoidal *Salmonella*, the chronic phase of this disease has remained difficult to study. Instead, researchers have used *S.* Typhimurium infections of the 129x1/svJ mouse model to study persistent infections, as *S.* Typhimurium can persist in tissues of these mice for at least one year [84]. Data from long term studies in 129x1/svJ mice indicate that there is a shift from T_H1 driven response at early time points, characterized by IL-1β, TNFα, IFNγ and IL-12 production, to T_H2 driven responses during persistent infection [100]. Although there are no proteomic studies on the cytokine

profile in human carriers of typhoidal *Salmonella*, several studies have demonstrated that acute typhoid fever is also accompanied by high serum levels of pro-inflammatory cytokines including IFNγ, IL-1, IL-6 and IL-8 [101-104]. While the cytokine response during the persistent phase of typhoid fever is currently unclear, some case reports suggest that children with prolonged *Mycobacterium* and *Salmonella* co-infection have reduced IFN-receptor- or IL-12 receptor expression in isolated blood cells, suggesting that functional interferon signaling is essential for the host to counteract long-term *Salmonella* infection [105-107].

One key feature of persistent *Salmonella* infections is the formation of granulomas, a structure formed to contain macrophages infected by *Salmonella* that cannot be cleared by the host. *Salmonella* driven formation of granulomas has been found in the MLN, liver and spleen in humans infected with typhoid fever, and thus granulomas may represent another mechanism by which the host influences *Salmonella* persistence [108-110]. These granuloma structures have also been observed during *S.* Typhimurium infection of 129x1/SvJ mice, and in this chronic infection model, granulomas form rapidly, with observable structures found by 1 week post infection [111]. Splenic granulomas are characterized by a lack of T_H1 cells, which allows *Salmonella* to persist and replicate in the activated macrophages [112]. Recent studies have also shown that *S.* Typhimurium facilitates the use of the effector protein SteE to polarize macrophages into an M2-like, anti-inflammatory phenotype within splenic granulomas, allowing *Salmonella* to persist within these structures during long-term infection [111,113]. Intriguingly, there is no homolog of *steE* encoded in the genomes of typhoidal *Salmonella*, and thus more research is needed to identify typhoidal-specific effectors that allow typhoidal *Salmonella* to persist within human granulomas.

5. Conclusion

As extensively antibiotic-resistant outbreaks of typhoid and paratyphoid fever increase in frequency and magnitude, a deeper understanding of human typhoidal disease is critical for the development of new therapies and vaccines. Identifying specific molecular mechanisms unique to typhoidal pathogenesis is key to developing anti-virulence strategies for targeted treatment. While much progress has been made in characterizing the molecular mechanisms that underlie *Salmonella* infections, especially in the context of non-typhoidal *Salmonella*, many questions remain. For example, while many genetic differences have been identified between non-typhoidal and typhoidal *Salmonella*, how do these changes in genotypes relate to differences in phenotype or disease manifestation? How do typhoidal strains of *Salmonella* replicate within human macrophages, and are there typhoid-specific virulence factors that play a role in these infections? What are the specific molecular determinants that allow typhoidal *Salmonella* to persist in human hosts for years, and are there better ways that we can model these human-adapted infections? Overall, by studying human-restricted typhoidal *Salmonella* in a variety of human-associated model systems, including human macrophages, human-derived intestinal organoids, and humanized mice, researchers may uncover human-specific mechanisms of virulence. In addition, understanding the human immune response to these “stealth” pathogens will likely reveal novel mechanisms of immune modulation.

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Highlights

- *Salmonella enterica* is a bacterial pathogen that is comprised of non-typhoidal and typhoidal isolates, aka "serovars".
- While non-typhoidal *Salmonella* causes self-limiting gastroenteritis, typhoidal *Salmonella* causes typhoid fever.
- The molecular mechanisms that underlie different disease manifestations are still being explored.
- In this review, we focus on the mechanisms that underlie the pathogenesis of typhoidal *Salmonella*.
- We highlight specific genetic traits that may contribute to typhoid fever, including serovar-specific virulence factors.
- We discuss how the host responds to infections by typhoidal vs. non-typhoidal *Salmonella*.

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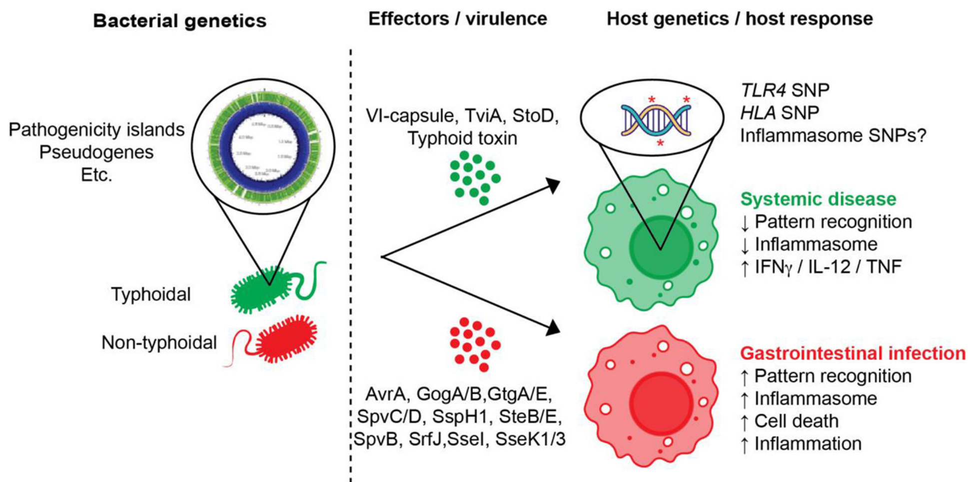

Figure 1. Summary figure highlighting some key known differences in the pathogenesis between typhoidal and non-typhoidal *Salmonella*.

Typhoidal and non-typhoidal serovars of *Salmonella* are genetically similar but have distinct features that lead to divergent disease phenotypes. For example, typhoidal and non-typhoidal serovars genomes differ in the repertoire of pathogenicity islands and pseudogenes that they contain. In addition, these genetic variations give rise to key differences in secreted effectors and virulence factors that influence the intracellular survival of *Salmonella* serovars. Finally, on the host side, SNPs in key genes that influence pattern recognition and antigen presentation may help typhoidal serovars evade local responses in the gastrointestinal tract and spread to systemic sites, triggering either enteric fever and/or chronic persistent infections.

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Table 1.

Horizontally Acquired Regions of the S. Typhi Ty2 Chromosome

Name	Length	Key Virulence Features
SPI-1	40,278 bp	T3SS-1; invasion, host cell modulation
SPI-2	39,633 bp	T3SS-2; intracellular replication, host cell modulation
SPI-3	36,845 bp	Magnesium uptake genes, many pseudogenes in *S.* Typhi
SPI-4	23,392 bp	SiiE adhesin & T1SS
SPI-5	7,408 bp	SopB, PipA, PipB
SPI-6	57,634 bp	T6SS; gut colonization & unique 10 kb in Typhi
SPI-7	131,750 bp	Unique to Typhi; capsule biosynthesis, SopEφ, type IVB pilus
SPI-8	-	(Unique 8kb part of SPI-13)
SPI-9	15,696 bp	T1SS, putative adhesin
SPI-10	33,675 bp	Unique to Typhi; contains ST46
SPI-11	10,080 bp	PhoP-activated genes, typhoid toxin
SPI-12	6,439 bp	SspH2
SPI-13	24,963 bp	An 8 kb portion is different between Typhi and Typhimurium
SPI-15	6,009 bp	Absent form Typhimurium, present in Typhi
SPI-16	4,087 bp	Three ORFs with a high level of identity with P22 phage genes
SPI-17	4,939 bp	Absent from Typhimurium, present in Typhi
SPI-18	1,678 bp	Absent from Typhimurium, present in Typhi; ClyA & TaiA
ST10	42,759 bp	Absent from Typhimurium, present in Typhi (prophage)
ST15	32,588 bp	Absent from Typhimurium, present in Typhi (prophage)
ST27	22,785 bp	Salmochelin iron acquisition operon (*iroCDEN*), PipB2, VirK
ST2-27	10,268 bp	Absent from Typhimurium, present in Typhi (prophage)
ST35	35,194 bp	Absent from Typhimurium, present in Typhi (prophage)
ST46	7,055 bp	Absent from Typhimurium, present in Typhi (prophage)
CS54	24,674 bp	RatA, SivI
SopEφ (within SPI-7)	33,971 bp	Present in some Typhimurium strains, present in Typhi Ty2 strain

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Table 2.

T3SS-Dependent Effectors in the Typhi Ty2 Genome

Effector	Ty2 Locus	CT18 Locus	*S*Tm LT2 Locus	Paratyphi A 9150 Locus	Locus Location	T3SS-1	T3SS-2
AvrA	⊘	⊘	STM2865	⊘	SPI-1	✓	✓
GogB	⊘	⊘	STM2584	⊘	Gifsy-1	✓	✓
GtgA	⊘	⊘	STM1026	⊘	Gifsy-2	✓	✓
GtgE	⊘	⊘	STM1055	⊘	Gifsy-2	✓	✓
PipB2	T_RS13590	STY2897	STM2780	SPA_RS13395	ST27	✓	✓
SlrP	T_RS10620	STY0833	STM0800	SPA_RS09800	-	✓	✓
SpvC	⊘	⊘	PSLT038	⊘	pSLT	✓	✓
SpvD	⊘	⊘	PSLT037	⊘	pSLT	✓	✓
SspH1	⊘	⊘	STM14_RS07010	⊘	Gifsy-3	✓	✓
SteA	T_RS07605	STY1482	STM1583	SPA_RS06440	-	✓	✓
SteB	⊘	⊘	STM1629	⊘	-	✓	✓
SteE (SarA)	⊘	⊘	STM2585	⊘	Gifsy-1	✓	✓
SipA	T_RS14125	STY3005	STM2882	SPA_RS13925	SPI-1	✓	
SipB	T_RS14140	STY3008	STM2885	SPA_RS13940	SPI-1	✓	
SipC	T_RS14135	STY3007	STM2884	SPA_RS13935	SPI-1	✓	
SipD	T_RS14130	STY3006	STM2883	SPA_RS13930	SPI-1	✓	
SopA	T_RS04065	STY2275	STM2066	SPA_RS04025	-	✓	
SopB	T_RS09305	STY1121	STM1091	SPA_RS08830	SPI-5	✓	
SopD	T_RS14435	STY3073	STM2945	SPA_RS14235	-	✓	
SopE	T_RS21905	STY4609	SL1344_RS13925	SPA_RS12970	SopEφ	✓	
SopE2	T_RS05225	STY1987	STM1855	SPA_RS05115	-	✓	
SopF	T_RS05655	STY1893	STM1239	SPA_RS08080	SPI-11	✓	
SptP*	T_RS14105	STY3001	STM2878	SPA_RS13905	SPI-1	✓	
StoD	T_RS09505	STY1076	⊘	⊘	ST10	✓	
GogA	⊘	⊘	STM2614	⊘	Gifsy-1		✓
CigR	T_RS19110	STY4024	STM3762	SPA_RS18310	SPI-3		✓
PipA	T_RS09330	STY1115	STM1087	SPA_RS08855	SPI-5		✓
PipB	T_RS09325	STY1117	STM1088	SPA_RS08850	SPI-5		✓
SifA	T_RS08635	STY1264	STM1224	SPA_RS08155	-		✓
SifB	T_RS07700	STY1462	STM1602	SPA_RS06345	-		✓
SopD2	T_RS09990	STY0971	STM0972	SPA_RS09170	-		✓
SpiC	T_RS06440	STY1727	SL1344_RS06920	SPA_RS07330	SPI-2		✓
SpvB	⊘	⊘	PSLT039	⊘	pSLT		✓
SrfA	T_RS07655	STY1472	STM1593	SPA_RS06390	-		Pred.
SrfB	T_RS07660	STY1471	STM1594	SPA_RS06385	-		Pred.
SrfC	T_RS07665	STY1470	STM1595	SPA_RS06380	-		Pred.
SrfJ	⊘	⊘	STM4426	⊘	-		✓
SseB	T_RS06465	STY1722	STM1398	SPA_RS07305	SPI-2		✓

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Effector	Ty2 Locus	CT18 Locus	*S*Tm LT2 Locus	Paratyphi A 9150 Locus	Locus Location	T3SS-1	T3SS-2
SseC	T_RS06475	STY1720	STM1400	SPA_RS07295	SPI-2		✓
SseD	T_RS06480	STY1719	STM1401	SPA_RS07290	SPI-2		✓
SseF	T_RS06495	STY1716	STM1404	SPA_RS07275	SPI-2		✓
SseG	T_RS06500	STY1715	STM1405	SPA_RS07270	SPI-2		✓
SseI (srfH)	⊘	⊘	STM1051	⊘	Gifsy-2		✓
SseJ	T_RS25885	STY_RS06750	STM1631	⊘	-		✓
SseK1	⊘	⊘	STM4157	⊘	-		✓
SseK2	T_RS23900	STY_RS11125	SL1344_RS10980	SPA_RS23070	-		✓
SseK3	⊘	⊘	SL1344_RS10000	⊘	ST64B		✓
SseL	T_RS02915	STY2517	SL1344_RS11745	SPA_RS02885	-		✓
SspH2	T_RS03160	STY2467	STM2241	⊘	SPI-12		✓
SteC	T_RS08195	STY1353	STM1698	SPA_RS05905	-		✓
SteD	T_RS03630	STY2367	STM2139	SPA_RS03565	-		✓

Grey box = Gene has a frameshift or early truncation in *S.* Typhi relative to *S.* Typhimurium, assumed to be non-functional

[*] = The *sptP* gene is mutated and non-functional in *S.* Typhi

⊘ = Gene is absent from this serovar

✓ = *S.* Typhimurium translocation of this effector is dependent on T3SS-1, −2 or both. "Pred." means predicted but not yet directly demonstrated.

If there is no homolog in S. Typhimurium str. LT2, homolog in strain SL1344 or 14028 given instead.