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# The mechanisms of virulence and antimicrobial resistance in *Salmonella enterica* serovar Typhi: A systematic review

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## Abstract

*Salmonella enterica* serovar Typhi is the etiologic agent of typhoid fever which is responsible for about 21,600 deaths annually, a large proportion of which is reported in developing countries. The organism is capable of evading the host defense mechanism to establish pathogenesis and this is enabled by the presence of specific virulence genes clustered in regions over the chromosome known as *Salmonella* Pathogenicity Island (SPI). Typhoid fever could be fatal therefore it requires effective antibiotic therapy. Strains which are antibiotic resistant could lead to increased mortality rates due to failure of routinely used antibiotics. This review gives an insight into the molecular mechanisms of virulence and antibiotic resistance so as to enhance more effective disease management and control.

***Keywords:*** *Salmonella enterica serovar Typhi, Enteric fever, Antibiotic resistance, Pathogenicity islands, Plasmids*



## 1. Introduction

*Salmonella* is a gram-negative, non-capsulated, non-spore forming rod that is motile with peritrichous flagella and possess outer coat antigens (Shahane *et al*., 2007). It is facultatively anaerobic with an optimum temperature and pH range of 37 °C and 6.5-7.5 respectively.

The major niche of *Salmonella* serovars is the intestinal tract of man and farm animals. Soil, feed, bedding, litter and faecal matter are the major sources of contamination in farms (Hoelzer *et al*., 2011; and Madoroba *et al*., 2016). They are common contaminants of a wide range of foods such as vegetables, water, milk, eggs, meat and meat products and thus considered as primary sources of food borne infection globally (Scallan *et al*., 2011; and Jajere *et al*., 2014).

*Salmonella* causes salmonellosis and enteric fever which is characterized by nausea, abdominal pain, diarrhoea and sometimes fever that result in morbidity and in some instances mortality in animals and man (Madoroba *et al*., 2016).

Salmonellae form a complex group of bacteria consisting of two species and six subspecies and 2,579 serovars (Malorny *et al*., 2011; and Hanning and Andino, 2015). Several species of this important group are pathogenic producing infections in many animal hosts and man. The genus *Salmonella* is comprised of two species, *S. bongori* and *S. enterica*. *S. enterica* is further subdivided into six subspecies each represented by roman

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numerals and a name: I, ssp. enterica; II, ssp. salamae; IIIa, ssp. arizonae; IIIb, ssp. diarizonae; IV, ssp. houtenae and VI, ssp. indica which are differentiated by biochemical characteristics and genomic phylogeny (Brenner *et al*., 2000; Perkins, 2009; and Kroft, 2017). Of the over 2,500 recognized serotypes, more than 1,500 are of subspecies I and associated with warm-blooded animals (Guibourdenche *et al*., 2010). *S. enterica* subspecies I strains are responsible for 99% of all reported human *Salmonella* infections in the United States (Eng *et al*., 2015; and Centers for Disease Control and Prevention, 2013). Serotyping, which utilizes variation of phase I (H1) and phase II (H2) flagellar and somatic lipopolysaccharide (O) antigens on the surface of bacterial cells, is commonly used to distinguish strains.

*Salmonella enterica is* a rod-shaped, flagellate, facultative aerobic, gram-negative bacterium and is a species of the genus *Salmonella*. *S. enterica* has serovars Typhi, Paratyphi, Enteritidis, Typhimurium and Choleraesius. A number of these serovars are serious human pathogens. Among more than 2,300 closely-related *Salmonella* serovars recognized, *S. enterica* serovar Typhi and Paratyphi are pathogenic exclusively for humans, and cause systemic infections and typhoid fever respectively whereas others such as *S. enterica* serovar Typhimurium cause gastroenteritis (Zhang *et al*., 2008). *S. enterica* serovar Typhimurium is frequently associated with gastroenteritis in humans, whereas this serovar causes a systemic typhoid-like disease in susceptible mice (Forest *et al*., 2010). There are two major groups of *S. enterica* that cause disease in humans: the systemic (typhoid fever) group and the non-typhoidal salmonellae group. Typhoid fever is mainly caused by *S. enterica* serovar Typhi (*Salmonella* Typhi), a host-adapted serovar that is specific for humans. Non-typhoidal salmonellae are generally associated with gastroenteritis, which rarely develops into an invasive infection.

*S. enterica* serovar Typhi is the causative agent of typhoid fever which is a systemic, life threatening disease of humans (Galan, 2016). *S. enterica* serovar Typhi is transmitted through contaminated food and water. Following ingestion of contaminated food or and water, the bacteria spread from the intestine via blood to the

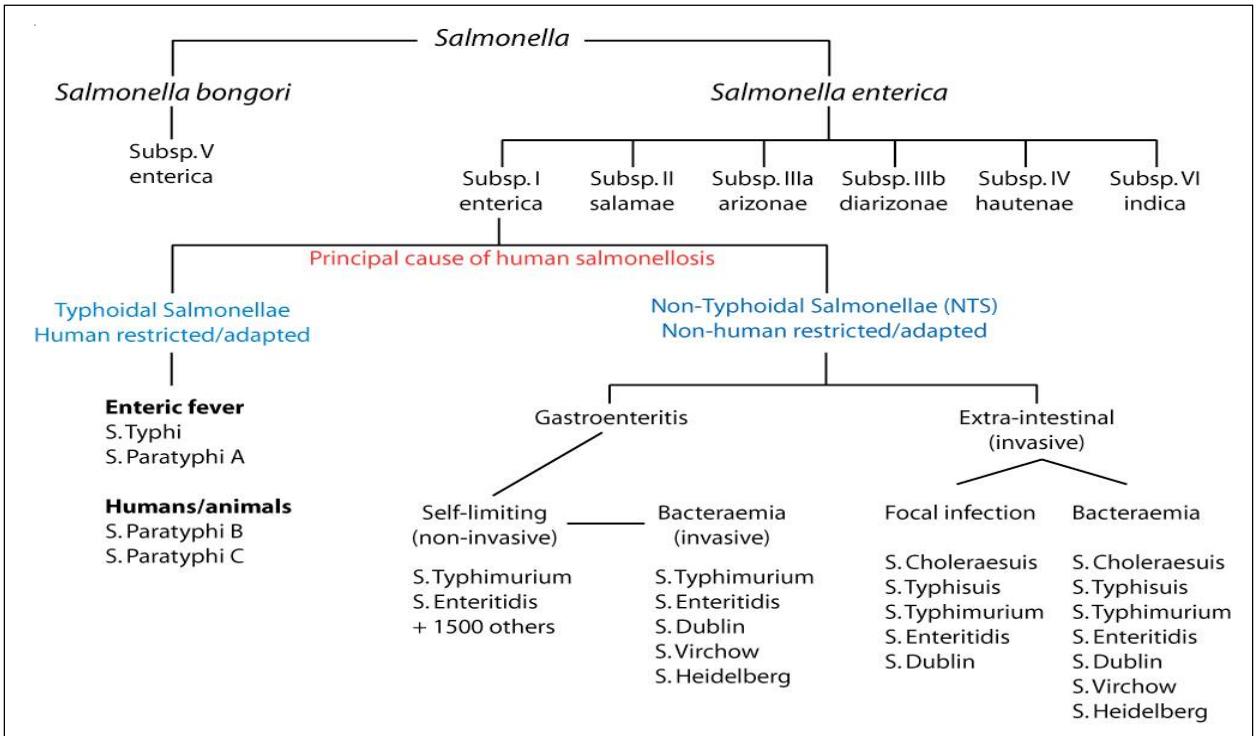


**Figure 1: Classification and diseases of *Salmonella* species (Perkins, 2009)**

intestinal lymph nodes, liver and spleen where they multiply (Kaur and Jain, 2012). A significant percentage of normal animals harbor *Salmonella* in their intestinal tracts (Jajere *et al*., 2014).

*S. enterica* serovar Typhi is a gram-negative, rod-shaped, flagellated bacterium whose only reservoir is the human body (Crump *et al*., 2015). It is the etiologic agent of an enteric fever called typhoid fever and considered a public health problem in the world (Fallah *et al*., 2016). They are intracellular pathogens with the ability of systemic spread and can also populate the intestinal lumen, causing diseases such as typhoid fever, blood infections and food borne gastroenteritis, depending on the host-pathogen-pairing.

As estimated, 16 to 22 million cases and about 21,600 deaths occur annually due to typhoid, most of which are reported in developing countries caused by consumption of unsafe drinking water and contaminated food.

It causes a severe systemic infection; typhoid fever, which is a serious worldwide public health problem. According to the World Health Organization (WHO), the annual global burden of typhoid fever is about 11-20 million new cases per year and 1% of which are fatal. More than 90% of typhoid fever cases occurred in Asia. It is highly prevalent in Asia and Africa due to shortage of hygienic water and poor sanitation. It is also a significant travel-associated disease (Connor and Schwartz, 2005). Therefore, *S. enterica* serovar Typhi infection poses substantial global disease burden on the healthcare system in endemic countries. Typhoid fever is clinically manifested through prolonged fever, abdominal discomfort, headache, and general lethargy (Liaquat *et al*., 2018).

## 2. Pathogenesis of *S. enterica* serovar Typhi

The infectious dose of *S. enterica* serovar Typhi varies between 1000-1,000,000 organisms. The incubation period is usually 7 to 14 days. On ingestion, after bypassing the gastric acidity, the organisms invade the intestinal epithelium through the Peyer's patches. After penetration, they translocate to the intestinal lymphoid follicles and mesenteric lymph nodes and even to the reticuloendothelial cells of the liver and spleen where they multiply and reach the blood stream referred to as "primary blood stream invasion". The bacteria get seeded in several reticuloendothelial sites and spill over from these sites into the bloodstream causing secondary bacteraemia and the patient now begins to exhibit symptoms. The organisms are then widely disseminated into liver, spleen, bone marrow, gall bladder and Peyer's patches of terminal ileum (Raveendran *et al*., 2010). However, the initial spread of the pathogen does not evoke overt host responses, as indicated by the fact that typhoid fever has an average incubation period of two weeks (Olsen *et al*., 2003). Pathological changes in the intestine are characterized by a slow development of inflammatory infiltrates that are dominated by mononuclear cells (macrophages and dendritic cells) while neutrophils are scarce (Sterzenbach *et al*., 2013). The organism spreads to internal organs, most frequently the bone marrow, the liver and the spleen where it is found in histiocytic granulomas, known as typhoid nodules (Sterzenbach *et al*., 2013). Spread to the gall bladder or urinary bladder can lead to chronic carriage, which is important for human-to-human spread of the disease.

Symptoms of typhoid fever are non-specific, commonly including fever and a slowed heart rate (bradycardia). Splenomegaly, hepatomegaly, or rose spots are encountered less frequently (Sterzenbach *et al*., 2013). Unlike gastroenteritis, typhoid fever is not considered a diarrhoeal disease, because this symptom develops late, after the onset of fever, in only a fraction (approximately one-third) of typhoid fever patients, while the remaining individuals remain either diarrhoea free or become constipated. *Salmonella* Paratyphi A, and less frequently S. Paratyphi B and Paratyphi C, are associated with paratyphoid fever, a disease that is milder in its course but otherwise indistinguishable from typhoid fever. Together with *S. enterica* serovar Typhi, these pathogens are commonly referred to as typhoidal *Salmonella* serotypes.

## 3. Pathogenesis elucidated by typhoidal toxin

Once the typhoid toxin is synthesized by *S. enterica* serovar Typhi, it is secreted into the lumen of the bacteria containing vacuole by a specific and unique protein secretion system although, the full mechanism is not yet fully understood (Hodak and Galán, 2013). The typhoid toxin is then packaged into vesicle carrier intermediate and subsequently transported to the extracellular space where it can reach its target host cells via paracrine or autocrine pathways4. o target host cells is not yet fully understood but is believed to be mediated by the enzymes Rab GTPases Rab 29 and Rab 31 (Galan, 2016). Typhoid toxin cannot intoxicate the cells that produced it until it becomes extracellular thus cells lacking typhoid toxin receptors and harboring *S. enterica* serovar Typhi could serve as a toxin source while themselves are not a target of toxicity.

## 4. Pathogenicity of *S. enterica serovar Typhi*

### 4.1. Salmonella *Pathogenicity Island (SPI)*

The ability of *Salmonella* Typhi to infect the host relies on the genetic determinants called virulence genes, located in the SPI (Zishiri *et al*., 2016). Pathogenicity islands are distinct genetic components located on the pathogenic bacterial chromosomes (Liaquat *et al*., 2018). The pathogenicity island is located either on the bacterial chromosome or on large virulence-associated plasmids. SPIs are portions of DNA that have been acquired from other microorganisms by horizontal gene transfer and they are absent in non-pathogenic strains

(Zishiri *et al*., 2016). SPI are characterized by a base composition different from the core genome and are often associated with tRNA genes and mobile genetic elements, like IS elements, transposons or phage genes. By now, 15 SPIs have been identified in *S. enterica serovar Typhi* (Vernikos and Parkhill, 2006). The SPIs also produces and excretes a protein known as invasin that allows non-phagocytic cells to take up the bacterium, where it is able to live intracellularly. It is also able to inhibit the oxidative burst of leukocytes, making innate immune response ineffective (Ugboko and De, 2014). Many of the *Salmonella* virulence factors, such as adhesion, invasion, and toxin genes are clustered in the SPI (Kaur and Jain, 2012). Currently, at least sixty (60) virulence genes associated with SPIs have been mapped so far and they all serve different functions (Zishiri *et al*., 2016). Some facilitate colonization of the pathogen to survive host defense mechanisms while some are responsible for multiplication inside the host. The SPIs for *S. enterica* serovar Typhi include SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-6, SPI-7, SPI-8, SPI-9, SPI-10, SPI-15, SPI-16 and SPI-17.

SPI-1 and SP1-2 are the most studied and are solely responsible for pathogenicity and they encode the genetic information for a large number of proteins referred to as type III secretion system (T3SS) (Moest and Meresse, 2013). There are two types of T3SS namely T3SS-1 and T3SS-2 which are encoded on SP1 and SP2 respectively. Both T3SSs play a role with regard to interactions with the host during pathogenesis. T3SS-1 facilitates the invasion of non-phagocytic cells and contributes to the crossing of epithelia. T3SS-2 is required for bacterial replication inside many eukaryotic cell types of the various organs reached during the development of a systemic infection (Moest and Meresse, 2013). *Salmonella* successively needs T3SS-1 and T3SS-2 to first invade the intestinal epithelium and subsequently survive in tissue phagocytes. T3SS-1 is mainly responsible for invasion, a process mediated by re-arrangement and polymerization of actin, leading to membrane ruffling and engulfment of the bacteria. It is also responsible for intestinal inflammation. Both SPIs seem to be important for different stages of the infectious life cycle of *S. enterica* serovarTyphi.

SPI-1 enables *S. enterica* serovarTyphi to initiate the infection and to invade the intestinal tract for further dissemination in the host. Genes encoded by this region are said to be essential in the invasion stage through the intestinal epithelium. Genes located in SPI-1 encode for several proteins (effector molecules), which are involved in the invasion of epithelial cells by mediating cytoskeletal rearrangement. These effector molecules are translocated into the host cells by type III secretion system (T3SS-1), which is composed of several operons. The prg/org and inv./spa operon encode the effector protein. SPI-2 mainly contributes to replication and survival of bacteria inside the host cell (epithelial cell and macrophages). SPI-2 mainly contains four groups of genes contributing to the virulence of *Salmonella*: *ssa*, the gene encoding for T3SS-2, *ssr* encoding for regulators, *ssg*: encoding the chaperones and *ssc* encoding the effectors. SPI-2 has been shown to be required for the survival of the pathogen within macrophages (Saxena *et al*., 2018). SPI-2 is composed of two regions; the larger region is responsible for systemic pathogenesis and it encodes for the second type of type three secretion system while the smaller region encodes the tetrathionate reductase (Ttr) which is responsible for anaerobic respiration (Khule and Hensel, 2004). SPI-3 encodes for proteins which are involved in both initial attachment and long-term persistence and survival during systemic phase of infection. This island allows *Salmonella* to adapt to environments where there is limiting nutritional requirement and is also involved in adhesion of *Salmonella* to host epithelial cells. SPI-3 is conserved in *S. enterica* serovar Typhi (Saxena *et al*., 2018). SPI-3 harbors 10 open reading frames amongst which is the *mgtC* gene. This gene is required by the organisms for growth in a $mg^{2+}$ limiting environment such as in phagosomes and also necessary for intramacrophage survival (Aphons *et al*., 2005). SPI-4 is required for intra-macrophage survival and is suspected to carry a type I secretion system (T1SS) involved in toxin secretion (Aphons *et al*., 2005). SPI-5 encodes the effector proteins for both the T3SS encoded by SPI-1 and SPI-2. It also carries the *sopB* gene which encodes an effector protein. SPI-5 is involved in accomplishing several pathogenic proven during infection (Nieto *et al*., 2016; and Riquelme *et al*., 2016). This island also encodes for Pip A and Pip B. Pip A contributes in the development of systemic infection while Pip B is involved in the accumulation of lipid rafts (Saxena *et al*., 2018). *saf* and *pag N* genes are borne on the SPI-6 and they encode for fimbriae and invasion protein respectively (Saxena *et al*., 2018). Vi antigen (capsular exopolysaccharide) is encoded by genes on SPI-7. This island also contains the *pil* gene cluster which encodes for putative factors. The genetic organization of SPI-7 is very complex and composed of several horizontally acquired elements. It contains few genes of conjugative plasmid-like *tra*and *sam.* The genes located in SPI-8 encode for putative virulence factors (Saxena *et al*., 2018). It also encodes for genes that confers resistance to bacteriocins (Aphons *et al*., 2005). SPI-9 encodes for type 1 secretion system (T1SS) and RTX (repeats in toxin) - like protein. SPI-10 contains a cryptic bacteriophage. Several virulence factors which contribute to *sef* fimbriae are also encoded on this island. SPI-11, SPI-12, SPI-13 and SPI-14 are not present in *S.*

**Table 1: Salmonella Pathogenicity Islands (SPI), genes on SPI and size of SPI, effector protein and their function**

| SPIs | Genes | Size of SPI | Effector protein synthesized | Function of effector protein | References |
|---|---|---|---|---|---|
| SPI-1 | *invA*, *prgI*, *hilA*, *sipA*, *prgH* | ≈40 kb | Sip A | Rearrangement of cytoskeletal system of non-phagocytic cells and recruitment of neutrophils | (Liaquat *et al.*, 2018; and Saxena *et al.*, 2018) |
| | | | Sip B | Nucleation of actin protein and translocation of other effector proteins/molecules | |
| | | | Sip C | Translocation of effector molecule | |
| | | | SOP A | Recruitment of immune cells and secretion of fluid in intestinal lumen | |
| | | | SOP C | Recruitment of neutrophils and secretion of fluid in intestinal lumen | |
| | | | SOP D | Recruitment of neutrophils and secretion of fluid in intestinal lumen | |
| | | | SOP E and spt P | Rearrangement of cytoskeletal of host cells | |
| | | | Iae P | Post translational modification of effector proteins of type III secretion system | |
| | | | Inv B | Act as chaperone | |
| | | | Avr A | Inhibition of apoptosis in epithelial cell, Inhibition of macrophage pyroptosis | |
| | | | Sic A Sic P | Act as chaperone | |
| SPI-2 | *spiC*, *sseB* | ≈40 kb | ssa B | Disruption of Golgi apparatus and Lysosomes, inhibition of SCV-lysosome fusion | (Liaquat *et al.*, 2018; and Saxena *et al.*, 2018) |
| | | | Ssa E | Acts as chaperone | |
| | | | Ssc A | Acts as chaperone | |
| | | | Ssc F | SCV perinuclear migration, microtubule bundling and SIF formation | |
| | | | Sse G | SCV perinuclear migration and SIF formation | |
| | | | Ttr genes | Tetrathionate respiration and outgrowth in the intestine | |
| | | | SPi C | Disruption of vesicular transport | |
| | | | SIF A | *Salmonella* containing vacuole membrane integrity | |

Table 1 (Cont.)

| SPIs | Genes | Size of SPI | Effector protein | Function of effector protein | References |
|---|---|---|---|---|---|
| | | | SsPH2 | Cytoskeleton rearrangements | |
| | | | SrFT | Apoptosis | |
| | | | Ssej | Cytoskeleton rearrangements | |
| | | | Pip B | Targeting to *Salmonella* induced filaments | |
| | | | SOP D2 | Targeting to *Salmonella* induced filaments/ late endosomes | |
| SPI-3 | *mgtB*, *mgtC*, *nepI/gaiA* | ≈17 kb | MgtC, MgtB (Magnesium transport system | Required for the adaptation of *Salmonella* in nutritional limitation conditions of the intra-phagosomal habitat | (Liaquat *et al.*, 2018; and Saxena *et al.*, 2018) |
| | | | MIS L | Anti-transport protein of SPI-3Allows for adhesion to host epithelial cells | |
| | | | Mar T | Activation of Mis L auto transport protein | |
| SPI-4 | *spi4d*, *orfL* | ≈27 kb | $T_1$SS | Putative virulence | (Saxena *et al.*, 2018) |
| | | | Sic E | Allows adhesion to epithelial cells | |
| SPI-5 | *pipA*, *pipB*, *pipD*, *sopB/sigD* | ≈7.6 kb | Ssr AB | Contributes to the development of systemic infection and accumulation of lipid rafts | (Saxena *et al.*, 2018) |
| SPI-6 | *tcf*, *safC*, *pag N* | ≈59 kb | Invasion protein | The genes; *saf* codes for fimbriae while *pag N* permits the invasion of host cells and tissues | (Saxena *et al.*, 2018) |
| SPI-7 | *pilS*, *tviA*, *tviB*, *tviD-E*, *sopE* | ≈133 kb | | Production of capsular exopolysaccharides and Vi antigen | (Saxena *et al.*, 2018) |
| SPI-8 | STY3280, STY3282 | ≈6.8 kb | | Responsible for putative virulence | (Saxena *et al.*, 2018) |
| SPI-9 | prtB, STY-2875 | ≈16 kb | $T_1$SSRTX-like protein | Responsible for toxin production and host cell invasion | (Aphons *et al.*, 2005) |
| SPI-10 | sefC, sefB, sefR, prpZ, prkY, prkX | ≈33 kb | | Production of Sef fimbriae | (Aphons *et al.*, 2005) |

*enterica* serovar Typhi (Saxena *et al.*, 2018). SPI-15 has an association with effector protein but its precise role is still unclear. SPI-16 and SPI-17 encode for proteins involved in lipopolysaccharide (LPS) modification (Saxena *et al.*, 2018). They also show association with tRNA genes.

### 4.2. *S. enterica* serovar Typhi toxin

Toxin production by *S. enterica* serovar Typhi remained undetectable for more than a century because the toxins are not liberated into the medium when cultured and could not be identified in culture supernatants or

cell lysates because intoxication was dependent on the ability of *S. enterica* serovar Typhi to invade or infect cultured cells and to transit to the endocytic pathway for a minimum of 3 h as to receive environmental cues that will culminate in the stimulation of toxin gene expression.

The typhoid toxin is also known as *Salmonella* cytolethal distending toxin (S- CDT) (Miller *et al*., 2018). The toxin induces DNA damage in eukaryotic cells. The typhoid toxin was discovered by Galan in his laboratory in Yale University. He identified a toxic activity exhibited by *S. enterica* serovar Typhi similar to that seen in other cells intoxicated with an exotoxin known as CDT. Similar to cells intoxicated with CDT, *S. enterica* serovar Typhi infected cells seemed distended with its nuclei doubling its normal size (Galan, 2016).

Toxicity is associated with the protein CDT which is encoded by the gene with homology to the *CdtB* gene located within the *Salmonella* genomic islets (SPI). S-CDT is an $A_2B_5$ toxin composed of a pentameric ring (Miller *et al*., 2018). This protein has a DNAse activity that is capable of damaging the DNA of eukaryotic cells (Galan, 2016). In the genomic islet where *CdtB* gene is located, open reading frames (ORFs) s encoding homologs of the adenosine diphosphate (ADP) ribosyl transferase "A" subunit and one of the components of the heteromeric "B" subunit of the pertussis toxin named as PltA and PltB respectively are also located or identified. Mutations in PltA or PltB genes can result in a total loss of the CdtB dependent toxicity. Also, CdtB, PltA and PltB have been observed to form a complex to cause toxicity (Figure 1) (Spanò *et al*., 2008). It is also believed that the typhoid toxin evolved from the exotoxin ancestors; CDT and pertussis toxins (Galan, 2016).

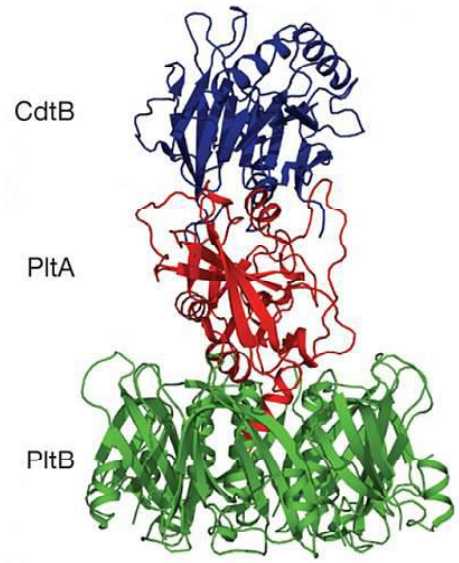


**Figure 2: Complex of typhoidal toxin showing the 2 A subunits (blue and red) and 5 B subunits (green) (Song *et al*., 2013)**

The toxin when elaborated into the eukaryotic cell accumulates in the G2/M cell cycle phase and activates a DNA damage response in the host cell (Miller and Wiedmann, 2016). The administration of purified S-CDT in vivo into a mouse model illicit partial recapitulated signs of typhoid fever (Gao *et al*., 2017).

An exotoxin which is heat labile known as *Salmonella* enterotoxin (Stn) encoded by the *stn* gene has been reportedly produced by *S. enterica* serovar Typhi strains as detected by PCR and Southern blotting (Aphons *et al*., 2005). The exact role of Stn in pathogenicity is not vivid enough however, there are indications that it plays a role in elevating cyclic adenosine monophosphate (cAMP) concentrations and increasing the synthesis and release of prostaglandins although the exact pathways leading to fluid and electrolyte secretion is not yet understood (Aphons *et al*., 2005).

### *4.3. Flagella and fimbriae*

Fimbriae are structures found on the cell surface of some bacteria, which have been shown to play an important role in the formation of biofilms, colonization, and the initial attack of the bacterium on the host (Santos *et al*., 2019).

The flagellum is a long helical filament coupled to rotating motors embedded within the outer membrane and cell wall, which enables ST and the other bacteria that display this feature to mobilize through the

epithelial barrier after ingestion. It is characterized as a strong inflammation inducer, mainly from the induction of Interleukin (IL)-8 and activation of NF-$\kappa\beta$, a protein complex that plays the role of a transcription factor and is involved in the immune response to infection. This induction is due to its chemotactic potential, which is also one of the main flagella characteristics (Santos *et al*., 2019).

### *4.4. Vi antigen*

*S. enterica* serovar Typhi expresses a capsular polysaccharide antigen called Vi antigen (Wain *et al*., 2005). The Vi antigen is a linear polymer of $\alpha$-1,4 2-deoxy-2-N-acetylgalacturonic acid that is variably O acetylated at the C-3 position (Tran *et al*., 2010). The Vi capsular antigen is a significant virulence factor for typhoid fever, as strains positive for Vi production have higher rates of infection (Tran *et al*., 2010). The Vi capsule inhibits phagocytosis and confers serum resistance (Johnson *et al*., 2018; and Hart *et al*., 2016), likely by shielding the O-antigen from antibodies (Hart *et al*., 2016). The genes encoding the Vi capsule comprise the *viaB* locus within the SPI-7, which also encodes the type III secretion system (T3SS) effector SopE and a type IVB pilus (Johnson *et al*., 2018). Vi expression is down regulated in the intestine, where flagella and SPI-1 play a role in invasion of epithelial cells, whereas it is upregulated in tissues during systemic dissemination, where it prevents antibody—mediated induction of neutrophil responses (Hiyoshi *et al*., 2018). Vi production is under the control of TviA, which induces expression of the Vi-encoding locus named *viaB*. Vi has been reported to bind cell surface prohibit in, thus dampening inflammation through MAPK signaling and IL-8 production (Sharma and Qadri, 2004). Reduced TLR5-and TLR4-mediated secretion of IL-8 leads to low levels of neutrophil influx (Figure 1), which is one of the characteristics of *S. enterica* serovar Typhi infection that make it distinct from the *S. enterica* serovar Typhimurium (Johnson *et al*., 2018).

Volunteer studies have indicated that Vi-positive strains of serovar Typhi are more virulent in humans than Vi-negative isolates, although Vi production is not essential for the infection process in humans (Liaquat *et al*., 2018).

### *4.5. S. enterica serovar Typhi virulence plasmids*

*S. enterica* serovar Typhi possesses plasmids which carry virulence genes and antimicrobial resistance genes (Kaur and Jain, 2012). The virulent plasmids have sizes varying from 50-90 kb and they share the *spv* operon, which is important for systemic infection (Lobato-Márquez *et al*., 2016). The *spv* genes appear to be important for bacterial multiplication within host cells during extra-intestinal infections (Guiney and Fierer, 2011). These virulence plasmids have a genetic locus called *Salmonella* plasmid virulence, which contains *spvRABCD* genes. It has been reported that the presence of *spv* genes greatly enhance the virulence of its host. Additional virulence genes located on virulence plasmids include those encoding fimbriae (*pef-BACDI*) and serum resistance; *traT* (Rotger and Casadesus, 1999). Although most virulence plasmids are not self-transmissible, some appear to contain a full concert of transfer (*tra*) genes that allow the plasmids to be transferred to additional strains by conjugation, thereby potentially increasing the virulence of the recipients (Ahmer *et al*., 1999). IncHI plasmids encode multiple-antibiotic resistance in *S. enterica* serovar Typhi. The plasmid R27 is considered the prototype of IncHI plasmids. These plasmids have been considered to play a relevant role in the persistence and re-emergence of this microorganism (Forns *et al*., 2005). Due to their conservation among members of a particular serovar, virulence plasmids provide a significant advantage to the strains that harbor these plasmids (Foley and Lynne, 2008). The plasmid pHCM1 also carries transferable genes for multidrug resistance in *S. enterica* serovar Typhi (Yan *et al*., 2016; and Wain *et al*., 2003). Plasmid $P^{R}_{ST98}$ is a large 159 kb plasmid isolated from *S. enterica* serovar Typhi strains from an outbreak of typhoid fever in the mid to late 1980s in China. Patients infected with *S. enterica* serovar Typhi carrying $P^{R}_{ST98}$ had more severe disease with a higher rate of complication and mortality. $P^{R}_{ST98}$ has been reported as a hybrid resistance-virulence plasmid carrying both antibiotic resistance and virulence genes. It has been suggested that these plasmids play important role in bacterial multiplication in the reticuloendothelial system of warm-blooded vertebrates although their exact role in pathogenesis is still not clear. Virulent plasmids affect intracellular growth in macrophages but not in phagocytic cells.

### *4.6. Phenotypic and genotypic mechanisms of antibiotic resistance in* S. enterica *serovar Typhi*

The routinely used antibiotics with bacteriostatic and bactericidal activity on *S. enterica* serovar Typhi include cotrimaxazole, fluroquinolone, trimethoprim-sulfomethoxazole, amoxicillin, ciprofloxacin, streptomycin, sulphonamides and tetracycline.

Chloramphenicol was the first antimicrobial used in the treatment of enteric fever. However, indiscriminate use of chloramphenicol led to the development of resistant *S. enterica* serovar Typhi within two years of its use (Raveendran *et al*., 2010). Chloramphenicol resistance was attributed to high molecular weight, self-transferable incompatibility complex group IncHI plasmids (Raveendran *et al*., 2010). Ampicillin, amoxicillin and cotrimoxazole became an alternative to chloramphenicol in the treatment of typhoid fever in the early 1970s. Resistance of *S. enterica* serovar Typhi to the first line antibiotics and different, newer antibiotics such as sulphonamides and tetracycline led to the emergence of strains called Multidrug-Resistant (MDR) strains. MDR *S. enterica* serovar Typhi is defined as *Salmonella* Typhi isolates which are resistant to three different classes of antimicrobials. However, the development of MDR strains that were resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and cotrimoxazole were identified towards the end of 1980s and 1990s. Consequently, the WHO recommended using third generation antibiotics, such as ciprofloxacin from the fluoroquinolone group. The fluoroquinolones (ciprofloxacin and ofloxacin), third generation cephalosporins (ceftriaxone and cefixime) and azithromycin were then used in treatment of MDR strains. Recently, *S. enterica* serovar Typhi that are MDR, non-susceptible to fluoroquinolones and resistant to third generation cephalosporins have evolved and are now known as Extensively Drug-Resistant (XDR) strains (Chatham-Stephens *et al*., 2019).

The recurrent use of the same antimicrobials against typhoid fever has led to an increase in multidrug resistance *S. enterica* serovar Typhi and other bacterial species. The use of first-line antimicrobials for the treatment of typhoid fever such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole has been recommended to be replaced with other antimicrobials such as ceftriaxone and cefotaxime.

Different studies have shown that the prevalence of antimicrobial resistance in *S. enterica* serovar Typhi is encoded by antimicrobial resistance—associated genes such as $bla_{SHV}$, $bla_{CMY-2}$, $bla_{TEM}$, *sul*, *aadA2*, *tetA*, *tetB*, *sul1*, *dfrA*, *floR*, *mphA*, Integron class I, *Cat* 1, *Cat* 2, *Cat3*, *cmlA*, *ant* (3″)-*Ia*, *strA*, *strB*.

*S. enterica* serovar Typhi produces Extended-spectrum $\beta$-lactamases (ESBLs): TEM, SHV and CTX-M which is encoded by $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$ genes respectively. Other genes coding for $\beta$-lactamases are $bla_{CMY}$, $bla_{PSE-1}$, $bla_{OXA}$. $\beta$-lactamases are enzymes that inactivates an antimicrobial, by cleave the four-membered ring in $\beta$-lactams. ESBLs are responsible for resistance of strains to third generation cephalosporins and ampicillin. The emergence of ESBL-producing *Salmonella* is a result of selective pressure imposed by the misuse of broad-spectrum antibiotics such as third-generation cephalosporins. $\beta$-lactamases are commonly found in gram-negative bacteria like *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* however, they have been isolated in *S. enterica* serovar Typhi and this has been attributed to the vertical transfer of the $\beta$-lactamase genes from other gram-negative bacteria to *S. enterica* serovar Typhi (Aljanaby and Medhat, 2017) and also a result of selective pressure imposed by the misuse of broad-spectrum antibiotics such as third-generation cephalosporins (Riyaaz *et al*., 2018). Tetracycline resistance genes (*tetA*, *tetB*, *tetG*) encode membrane associated efflux pump proteins that export tetracycline from the cell and reduces drug concentration and thereby protecting ribosomes. The plasmid mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrC*, *qnrS*) encode pentapeptide repeat proteins that bind to and protects DNA gyrase and topoisomerases IV from the inhibition of quinolones. The phenicol resistance genes, (*cat1*, *cat2*) encode chloramphenicol acetyltransferase enzyme that inactivates chloramphenicol, chloramphenicol resistance gene, *cmlA* and florfenicol resistance gene; *floR* encodes efflux pump proteins. Sulfonamide resistance genes sul1 and sul2 encode insensitive sulfonamide-resistant dihydropteroate synthase which cannot be inhibited by sulfonamide. Trimethoprim resistance genes (*dhfrI*, *dhfrV*, *dhfrVII*, *dhfrIX*, *dhfrXIII*) encode a drug-insensitive dihydrofolate reductase which cannot be inhibited by trimethoprim. Integrons are genetic elements that are able to recognize and capture mobile gene cassettes carrying the antibiotic resistance genes, which leads to MDR distribution and subsequently limits the available treatment options for infectious diseases (Abdel Aziz *et al*., 2018). Class 1 and 2 integrons were identified in *Salmonella*, and class 1 is the most predominant (Abdel Aziz *et al*., 2018). Class 1 integrons are known for their roles in the dissemination of AMR, especially in the carrying of multiple AMR genes (Odoch *et al*., 2018). Class I integrons may be localized in plasmids, which are mobile DNA elements that are important in the proliferation of MDR Gram-negative enteric bacteria and others. Conjugative transfer of bacterial plasmids cause acquired antimicrobial resistance phenotypes between different bacteria. Class I integrons are always associated with *sul1* genes (Odoch *et al*., 2018).

Multidrug resistance in *S. enterica* serovar Typhi is encoded for by large, self-transferable IncHI1 plasmids. IncHI1 plasmids exhibit resistance to nearly all commonly available antibiotics. Three fully sequenced IncHI1 plasmids have been recognized; R27, pHCM1, pAKU1 (Wain *et al*., 2009). A novel MDR plasmid has been

identified in *S. enterica* known as pSGB23 which harbors the incompatibility plasmids and carries 12 antimicrobial resistance genes which confers resistance to nine (9) classes of antibiotics and it is believed to carry the greatest number of antibiotic resistance genes with the broadest range of resistance spectrum among *S. enterica* MDR plasmid identified thus far. The plasmid has also been isolated from food (Ding *et al.*, 2018).

Colistin is a last-resort antibiotic for treatment of severe bacterial infections in critically ill patients caused by MDR and XDR bacteria. Colistin is not a routinely used antibiotic for the treatment of typhoid fever however due to the existence of multidrug and extensively resistant *S. enterica* serovar Typhi, there is a possibility that this antibiotic in the nearest future may be used in treatment of typhoid fever thus plasmid mediated colistin

**Table 2: Mechanism of antibiotic resistance genes (Odoch *et al.*, 2018)**

| Antibiotic | Antibiotic resistance genes | Resistance mechanism |
|---|---|---|
| β-lactams | *ompC*, *ompF*, $bla_{CMY}$-2, $bla_{PSE-1}$, $bla_{TEM}$-1, $bla_{SHV-1}$, $bla_{OXA-1}$, $bla_{NDM-1}$ | β-lactamases, ESBL, Modification of porin (*ompF*), Efflux of â-lactam (*ompC*) |
| Quinolones and fluoroquinolones/ Ciprofloxacin | *gyrA*, *gyrB*, *parC*, *parE*, qnrA, qnrB, qnrC, qnrS | Mutation in the Quinolones Resistance Determining Region (QRDR) *GryA*, *GyrB*, *parC*, *pare* |
| Aminoglycosides | *aacC(3)*, *aacC(3')-IIa*, *aacC(6')*, *aacC2*, *aadA*, *aadA1*, *aadA2*, *aadA12*, *aadB*, *ant(3")-Ia*, *aphAI*, *aphAI IAB*, *aph(3)-Ii-iv*, *aph(3)-IIa*, *strA*, *strB* | Enzymatic modification and inactivation of aminoglycoside |
| Tetracycline | *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(G)*, and *tet(H)* | Efflux pumps, Modification of rRNA target, Inactivation of compound |
| Sulfonamides | *Sul1*, *sul2 sul*3, *dfr* | Dihydropteroate synthase |
| Chloramphenicol | *floR*, *cmlA*, *cat1*, *cat2* | Efflux pumps (*floR*, *cmlA*) and chloramphenicol acetyltransferase |
| Trimethoprim | *dhfrI*, *dhfrV*, *dhfrVII*, *dhfrIX*, *dhfrXIII* | Integron-borne dihydrofolate reductases |
| Integron | Class 1 integron 3′- CS *aadA*, *veb-1*, $bla_{VIM-2'}$ 5′- CS Class 2 integron hep51 hep74 | Gene cassettes encoding for multidrug resistance |

resistance might compromise treatment of complicated enteric fever caused by *S. enterica* serovar Typhi. *mcr* (mobilized colistin resistance) genes encode resistance to MDR and XDR bacteria. Plasmid mediated colistin resistance conferred by *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 have already been identified in *S. enterica* serovars (Lima *et al.*, 2019). Recently, *mcr*-9 gene was discovered in *S. enterica* serovar Typhimurium (Caroll *et al.*, 2019). These genes are plasmid-borne genes and in *S. enterica* serovars they are borne on the plasmid IncHI2 (Da Silva *et al.*, 2019). Due to the relatedness between *S. enterica* serovar Typhi and *S. enterica* serovar Typhimurium, there is the possibility of a horizontal transfer of these genes to *S. enterica* serovar Typhi from *S. enterica* serovar Typhimurium.

## 5. Conclusion

The ability of *S. enterica* serovar Typhi to invade the host cells and disseminate in the body is closely related to its virulence genes. These genes are distributed in several regions over the chromosome known as SPI. There are about 13 SPIs in *S. enterica* serovar Typhi however, it has been observed that SPI-1 and SPI-2 contributes significantly to the virulence of *S. enterica* serovar Typhi because they carry the genes for invading the host intestinal epithelium and also to survive in the host intestine and macrophages. Their ability to also produce

toxins that is heat labile and capable of activating DNA damage response in the host cell also exacerbates its pathogenicity. It has been observed that the presence of integrons or plasmids shows that some of the virulent attributes were acquired from closely related pathogenic enteric organisms. The ineffectiveness of first, second and third generation antibiotics in the treatment of typhoid fever is also worrisome especially since fatality reports have been reported especially in developing countries where they are most prone to this disease due to consumption of contaminated food and water. The emergence of multidrug and extensively resistant strains underscores the urgency to seek for alternative preventive and curative measures in disease management. The horizontal transfer of mobile genetic elements such as integrons or plasmids carrying antibiotic resistance and virulence genes into the chromosome confers on *S. enterica* serovar Typhi strong antimicrobial resistance. Drugs could be designed to maneuvers the antibiotic resistance mechanisms employed by *S. enterica* serovar Typhi especially since the molecular mechanisms are known. Also, proper monitoring of drug prescription and use, improved diagnostics, prudent use of antimicrobials and, development and use of effective vaccines could prove very useful in reducing the global burden of the disease caused by *S. enterica* serovar Typhi and reducing the prevalence of MDR strains.

## References


Abdel Aziz, S. A., Abdel Latef, K. G., Shany, A. S. S. and Rouby, S. R. (2018). Molecular detection of integrin and antimicrobial substances genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-Suef governorate, Egypt. *Beni-Suef University Journal of Basic and Applied Sciences*, 7(4), 535-542.

Ahmer, B. M. M., Tran, M. and Heffron, F. (1999). The virulence plasmid of *Salmonella* typhimurium is self-transmissible. *Journal of Bacteriology*, 181, 1364-1368.

Aljanaby, A. A. J. and Medhat, A. R. (2017). Prevalence of some antimicrobial resistance associated-genes in *Salmonella typhi* isolated from patience infected with typhoid fever. *Journal of Biological Sciences*, 17, 171-184.

Aphons, J. A. M., van Asten, Jaap E. van Dijk (2005). Distribution of "classic" virulence factors among *Salmonella* spp. *FEMS Immunology and Medical Microbiology*, 44(3), 251-259.

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R. and Swaminathan, B. (2000). Salmonella nomenclature. *Journal of Clinical Microbiology*, 38(7), 2465-2467.

Caroll, L. M., Gaballa, A., Guldimann, C., Sullivan, G., Henderson, L. O. and Wiedmann, M. (2019). Identification of novel mobilized colistin resistance gene *mcr-9* in a multidrug-resistant, colistin-susceptible Salmonella enterica serotype typhimurium isolate. *MBio*, 10(3), e00853-19. doi: 10.1128/mBio.00853-19

Centers for Disease Control and Prevention (2013). An Atlas of *Salmonella* in the United States, 1968-2011: laboratory-based enteric disease surveillance, US Department of Health and Human Services, CDC, Atlanta, GA. https://www.cdc.gov/salmonella/pdf/schwarzengrund-508c.pdf

Chatham-Stephens, K., Medalla, F. and Hughes, M. (2019). Emergence of extensively drug resistant *Salmonella* Typhi infections among travellers to or from Pakistan – United States, MMR and morbidity mortality Weekly Report, 68, 11-13. doi: http://dx.doi.org/10.15585/mmwr.mm6801a3

Connor, B. A. and Schwartz, E. (2005). Typhoid and paratyphoid fever in travellers. *Lancet Infectious Disease*, 5, 623-628. pmid:16183516

Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. and Parry, C. M. (2015). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Reviews*, 28(4), 901-937.

Da Silva, G.J., Domingues, S. and Lima, T. (2019). Plasmid-mediated colistin resistance in *Salmonella enterica*: A review. Microorganisms, 7(2), 55. doi: 10.3390/microorganisms7020055

Ding, Y., Zwe, Y. H., Chin, S. F., Kohli, G. S., Drautz-Moses, D. I., Givskov, M., Schlundt, J., Schuster, S. C., Yuk, H. and Yang, L. (2018). Characterisation of a novel multidrug resistance plasmid pSGB23 isolated from *Salmonella* enterica subspecies enterica serovar Saintpaul. *Gut Pathogens*, 10, 20-24.

Eng, S., Pusparajah, P., Mutalib, N. A., Ser, H., Chan, K. and Lee, L. (2015). *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, 8(3), 284-293, doi: 10.1080/21553769.2015.1051243

Fallah, F., Godarzi, H., Lahoorpour, F., Bandehpour, M., Soleiman–Jahi, S. and Gachkar, L. (2016). Molecular and phenotypic characteristics of *Salmonella enterica* Serovar Typhi isolated from asymptomatic carrier. *Infection, Epidemiology and Medicine*, 2(3), 1-4.

Foley, S. L. and Lynne, A. M. (2008). Food animal-associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance. *Journal of Animal Science*, 86(E. Suppl.), E173-E187.

Forest, C. G., Ferraro, E., Sabbagh, S. C. and Daigle, F. (2010). Intracellular survival of *Salmonella enterica* serovar Typhi in human macrophages is independent of *Salmonella* pathogenicity island (SPI)-2. *Microbiology*, 156, 3689-3698. doi:10.1099/mic.0.041624-0

Forns, N., Baños, R. C., Balsalobre, C., Juarez, A. and Madrid, C. (2005). Temperature-dependent conjugative transfer of R27: Role of chromosome- and plasmid-encoded Hha and H-NS proteins. *Journal of Bacteriology*, 187(12), 3950-3959. doi: 10.1128/JB.187.12.3950-3959.2005

Galan, J. E. (2016). Typhoid toxin provides a window into typhoid fever and the biology of *Salmonella* Typhi. *Proceedings of the National Academy of Sciences*, 113(23), 6338-6344. doi:10.1073/pnas.1606335113

Gao, X., Deng, L., Stack, G., Yu, H., Chen, X., Naito-Matsui, Y., Varki, A. and Galan, J. E. (2017). Evolution of host adaptation in the *Salmonella* typhoid toxin. *Nature Microbiology*, 2, 1592-1599. doi: 10.1038/s41564-017-0033-2

Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P. I., Bockemuhl, J., Grimont, P. A. and Weill, F. X. (2010). Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Research in Microbiology*, 161, 26-29. doi: 10.1016/j.resmic.2009.10.002

Guiney, D. G. and Fierer, J. (2011). The role of the spv genes in *Salmonella* pathogenesis. *Frontiers in Microbiology*, 2, 129. doi:10.3389/fmicb.2011.00129

Hanning, I. and Andino, A. (2015). *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, Vol. 2015, Article ID 520179, 16 pages. https://doi.org/10.1155/2015/520179.

Hart, P. J., O' Shaughnessy, C. M., Siggins, M. K., Bobat, S., Kingsley, R. A., Goulding, D. A. and Maclennan, C. A. (2016). Differential killing of *Salmonella enterica* serovar Typhi by antibodies targeting VI and lipopolysaccharide O:9 antigen. *PLOS One*, 11(1), e0145945. https://doi.org/10.1371/jounal.pone.0145945

Hiyoshi, H., Wangdi, T., Lock, G., Saechao, C., Raffatellu, M., Cobb, B. A. and Bäumler, A. J. (2018). Mechanisms to evade the phagocyte respiratory burst arose by convergent evolution in typhoidal *Salmonella* serovars. *Cell Reports*, 22(7), 1787-1797. https://doi.org/10.1016/j.celrep.2018.01.016

Hodak, H. and Galán, J. E. (2013). A *Salmonella* Typhi homologue of bacteriophage muramidases controls typhoid toxin secretion. *EMBO Rep.*, 14(1), 95-102.

Hoelzer, K., Switt, A. I. M. and Wiedmann, M. (2011). Animal contact as a source of human non-typhoidal salmonellosis. *Veterinary Research*, 42(1).

Jajere, S. M., Onyilokwu, S. A., Adamu, N. B., Atsanda, N. N., Saidu, A. S., Adamu, S. G. and Mustapha, F. B. (2014). Prevalence of *Salmonella* infection in dogs in Maiduguri, Northeastern Nigeria. *International Journal of Microbiology*, Article ID 392548, http://dx.doi.org/10.1155/2014/392548

Johnson, R., Mylona, E. and Frankel, G. (2018). Typhoidal *Salmonella:* Distinctive virulence factors and pathogenesis. *Cellular Microbiology*, 20, e12939, 1-14.

Kaur, J. and Jain, S. K. (2012). Role of antigens and virulence factors of *Salmonella enterica* serovar Typhi in its pathogenesis. *Microbiological Research*, 167(4), 199-210. https://doi.org/10.1016/j.micres.2011.08.001

Khule, V. and Hensel, M. (2004). Cellular microbiology of intracellular *Salmonella enterica*: Functions of the type III secretion system of encoded by *Salmonella* pathogenicity island 2. *Cellular and Molecular Life Sciences*, 61, 2812-2826.

Kroft, B. S. (2017). Characterization, distribution and evolutionary acquisition of antimicrobial resistance elements among diverse *Salmonella* subspecies. Doctorate dissertation, Faculty of the Graduate School of the University of Maryland, College Park. pp. 1-16.

Liaquat, S., Sarwar, Y., Ali, A., Haque, A., Farooq, M., Martinez-Ballesteros, I., Lorena, L., Garaizar, J. and Bikandiet, J. (2018). Virulotyping of *Salmonella enterica* serovar Typhi isolates from Pakistan: Absence of complete SPI10 in Vi negative isolates. *PLoS Neglected Tropical Diseases*, 12(11), e0006839. https://doi.org/10.1371/journal. pntd.0006839

Lima, T., Domingues, S. and Da Silva, G. J. (2019). Plasmid-mediated colistin resistance in *Salmonella enterica*: A review. *Microorganisms*, 7(2), 55. doi:10.3390/microorganisms7020055

Lobato-Márquez, D., Molina-García, L., Moreno-Córdoba, I., García-del Portillo, F. and Díaz-Orejas, R. (2016). Stabilization of the virulence plasmid pSLT of *Salmonella* Typhimurium by three maintenance systems and its evaluation by using a new stability test. *Frontiers in Molecular Biosciences*, 3,66. doi: 10.3389/fmolb.2016.00066

Madoroba, E., Kapeta, D. and Gelaw, A. K. (2016). *Salmonella* contamination, serovars and antimicrobial resistance profiles of cattle slaughtered in South Africa. *Onderstepoort Journal of Veterinary Research*, 83(1), 1-8.

Malorny, B., Hauser, E. and Dieckmann, R. (2011). *New approaches in subspecies-level Salmonella classification*, in Porwollik, S. (Ed.), *Salmonella from Genome to Function*. pp. 1-23, Academic Press, Norfolk, UK.

Miller, R. A. and Wiedmann, M. (2016). The cytolethal distending toxin produced by nontyphoidal *Salmonella* serotypes Javiana, Montevideo, Oranienburg, and Mississippi induces DNA damage in a manner similar to that of serotype Typhi. *mBio*, 7, e02109-16. doi:10.1128/mBio.02109-16

Miller, R. A., Betteken, M. I., Guo, X., Altier, C., Duhamel, G. E. and Wiedmann, M. (2018). The typhoid toxin produced by the nontyphoidal *Salmonella* enterica serotype Javiana is required for induction of a DNA damage response in vitro and systemic spread in vivo. *mBio*, 9, e00467-00418. doi: 10.1128/mBio.00467-18

Moest, T. P. and Meresse, S. (2013). *Salmonella* T3SSs: successful mission of the secret(ion) agents. *Current Opinion in Microbiology*, 16, 38-44.

Nieto, P. A., Pardo-Roa, C., Salazar-Echegarai, F. J., Tobar, H. E., Coronado, I., Riedel, C. A., Kalergis, A. M. and Bueno, S. M. (2016). New insights about excisable pathogenicity islands in *Salmonella* and their contribution to virulence. *Microbes and Infection*, 18(5), 302-309. doi: 10.1016/j. micinf.2016.02.001

Odoch, T., Sekse, C., Abee-Lund, T. M., Hansen, H. H. C., Kankya, C. and Wasteson, Y. (2018). Diversity and antimicrobial resistance genotypes in non-typhoidal *Salmonella* isolates from poultry farms in Uganda. *International Journal of Environmental Research and Public Health*, 15(2), 324-338. https://doi.org/10.3390/ijerph15020324

Olsen, S. J., Bleasdale, S. C., Magnano, A. R., Landrigan, C., Holland, B. H., Tauxe, R. V., Mintz, E. D. and Luby, S. (2003). Outbreaks of typhoid fever in the United States, 1960-99. *Epidemiology and Infection*, 130, 13-21.

Perkins, T. T. (2009). Characterisation of the transcriptome and proteome of *Salmonella enterica* subspecies *enterica* serovar Typhi. Doctorate dissertation, Jesus College. 16-54.

Raveendran, R., Datta, S. and Wattal, C. (2010). Drug resistance in Salmonella enteric serotype Typhi and Paratyphi A. *Journal International Medical Sciences Academy*, 23(1), 21-24.

Riquelme, S., Varas, M., Valenzuela, C., Velozo, P., Chahin, N., Aguilera, P., Sabag, A., Labra, B., Álvarez, S. A., Chávez, F. P. and Santiviago, C. A. (2016). Relevant genes linked to virulence are required for *Salmonella* Typhimurium to survive intracellularly in the social amoeba *dictyosteliumdiscoideum*. *Frontiers in Microbiology*, 7, 1305. doi: 10.3389/fmicb.2016.01305

Riyaaz, A. A. A., Perera, V., Sivakumaran, S. and de Silva, N. (2018). Typhoid fever due to extended spectrum *b*-lactamase-producing *Salmonella enterica* serovar Typhi: A case report and literature review. *Case Reports in Infectious Diseases*. 2018, 2018-2023. https://doi.org/10.1155/2018/4610246

Rotger, R. and Casadesus, J. (1999). The virulence plasmids of *Salmonella*. *International Journal of Microbiology*, 2, 177-184.

Santos, A. D., Ferrari, R. G. and Carlos, C. J. (2019). Virulence factors in *Salmonella* Typhimurium: The sagacity of a bacterium. *Current Microbiology*, 76(6), 762-773. 10.1007/s00284-018-1510-4

Saxena, M. K., Kumar, R., Saxena, A. and Singh, Y. (2018). *Virulence System of Salmonella with Special Reference to Salmonella enterica.* In M. T. Mascellino (Ed.). *Salmonella- A Re-Emerging Pathogen.* London, IntechOpen, UK. 41-53.

Scallan, E., Hoekstra, R. M. and Angulo, F. J. (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Diseases,* 17(1), 7-15.

Shahane, V., Muley, V., Kagal, A. and Bharadwaj, R. (2007). Non-typhoid Salmonellosis: Emerging infection in Pune. *Indian Journal of Medical Microbiology,* 25, 173-174. doi: 10.4103/0255-0857.32734

Sharma, A. and Qadri, A. (2004). Vi polysaccharide of *Salmonella* Typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory responses. *Proceedings of the National Academy of Sciences of the United States of America,* 101(50), 17492-17497. https://doi.org/10.1073/pnas.0407536101

Song, J., Gao, X. and Galan, J. E. (2013). Structure and function of the *Salmonella* Typhi chimaericA(2)B(5) typhoid toxin. *Nature,* 18,499(7458), 350-354. doi: 10.1038/nature12377

Spanò, S., Ugalde, J. E. and Galán, J. E. (2008). Delivery of a *Salmonella* Typhi exotoxin from a host intracellular compartment. *Cell Host Microbe,* 3(1), 30-38.

Sterzenbach, T., Bäumler, A. J., Winter, S. E. and Crawford, R. W. (2013). *Salmonella* virulence mechanisms and their genetic basis. In Barrow, P. A. and Methner, U. (Eds). *Salmonella in Domestic Animals.* (2nd ed.). CABI, Oxfordshire, UK. p. 81.

Tran, Q. T., Gomez, G., Khare, S., Lawhon, S. D., Raffatellu, M., Baumler, A. J., Ajithdoss, D., Dhavala, S. and Adams, L. G. (2010). The *Salmonella enterica* serotype Typhi Vi capsular antigen is expressed after the bacterium enters the Ileal Mucosa. *Infection and Immunity,* 78(1), 527-535. doi:10.1128/IAI.00972-09

Ugboko, H. and De, N. (2014). Mechanisms of antibiotic resistance in *Salmonella* typhi. *International Journal of Current Microbiology and Applied Sciences,* 3(12), 461-476.

Vernikos, G. S. and Parkhill, J. (2006). Interpolated variable order motifs for identification of horizontally acquired DNA: revisiting the *Salmonella* pathogenicity islands. *Bioinformatics,* 22, 2196-2203.

Wain, J., Diem Nga, L. T., Kidgell, C., James, K., Fortune, S., Song Diep, T. and Dougan, G. (2003). Molecular analysis of incHI1 antimicrobial resistance plasmids from *Salmonella* serovar Typhi strains associated with typhoid fever. *Antimicrobial Agents and Chemotherapy,* 47(9), 2732-2739. doi:10.1128/aac.47.9.2732-2739.2003

Wain, J., House, D., Zafar, A., Baker, S., Nair, S., Kidgell, C., Bhutta, Z., Dougan, G. and Hasan, R. (2005). Vi antigen expression in *Salmonella enterica* serovar typhi clinical isolates from Pakistan. *Journal of Clinical Microbiology,* 43(3), 1158-1165. doi: 10.1128/JCM.43.3.1158-1165.2005

Wain, J., Phan, M., Kidgell, C., Nair, S., Holt, K. E., Turner, A. K., Hind, J., Butcher, P., Cooke, F. J., Thomson, N. R., Titball, R., Bhutta, Z. A., Hasan, R., Dougan, G. and Phan, M. (2009). Variation in *Salmonella enterica* serovar Typhi INcHI1 plasmids during the global spread of resistant typhoid fever. *Antimicrobial Agents and Chemotherapy,* 53(2), 716-727.

Yan, M., Li, X., Liao, Q., Li, F., Zhang, J. and Kan, B. (2016). The emergence and outbreak of multidrug-resistant typhoid fever in China. *Emerging Microbes & Infections,* 5(6), e62. doi:10.1038/emi.2016.62

Zhang, X., and Jeza, V. T. and Pan, Q. (2008). *Salmonella* Typhi: from a human pathogen to a vaccine vector. *Cellular & Molecular Immunology,* 5, 91-97. 10.1038/cmi.2008.11

Zishiri, O. T., Mkhize, N. and Mukaratirwa, S. (2016). Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort Journal of Veterinary Research,* 83(1), 11. doi: https://doi.org/10.4102/ojvr.v83i1.1067

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