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

Fowoyo P. T.*

1Microbiology Department, Baze University, Abuja, Nigeria. E-mail: patience.fowoyo@bazeuniversity.edu.ng

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...
Numerals and a name: I, sspp. enterica; II, sspp. salamae; IIIa, sspp. arizonae; IIIb, sspp. diarizonae; IV, sspp. houtenae and VI, sspp. 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 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.

Figure 1: Classification and diseases of Salmonella species (Perkins, 2009)
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 pathways. The 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.
SPI-1 and SPI-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 SPI1 and SPI2 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 serovar Typhi.

SPI-1 enables S. enterica serovar Typhi 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-1 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, ssf 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 mg2+ 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 1 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 pagN 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 confer 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 srf fimbriae are also encoded on this island. SPI-11, SPI-12, SPI-13 and SPI-14 are not present in S.
<table>
<thead>
<tr>
<th>SPIs</th>
<th>Genes</th>
<th>Size of SPI</th>
<th>Effector protein synthesized</th>
<th>Function of effector protein</th>
<th>References</th>
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<tbody>
<tr>
<td>SPI-1</td>
<td>invA, prgI, hilA, sipA, prgH</td>
<td>≥40 kb</td>
<td>Sip A</td>
<td>Rearrangement of cytoskeletal system of non-phagocytic cells and recruitment of neutrophils</td>
<td>(Liaquat et al., 2018; and Saxena et al., 2018)</td>
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<td></td>
<td></td>
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<td>Sip B</td>
<td>Nucleation of actin protein and translocation of other effector proteins/molecules</td>
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<td></td>
<td>Sip C</td>
<td>Translocation of effector molecule</td>
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<td>SOP A</td>
<td>Recruitment of immune cells and secretion of fluid in intestinal lumen</td>
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<td>Recruitment of neutrophils and secretion of fluid in intestinal lumen</td>
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<td>SOP E and spt P</td>
<td>Rearrangement of cytoskeletal of host cells</td>
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<td>Iae P</td>
<td>Post translational modification of effector proteins of type III secretion system</td>
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<td></td>
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<td>Inv B</td>
<td>Act as chaperone</td>
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<td></td>
<td></td>
<td>Avr A</td>
<td>Inhibition of apoptosis in epithelial cell, Inhibition of macrophage pyroptosis</td>
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<td></td>
<td>Sic A Sic P</td>
<td>Act as chaperone</td>
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<tr>
<td>SPI-2</td>
<td>spiC, sseB</td>
<td>≥40 kb</td>
<td>ssa B</td>
<td>Disruption of Golgi apparatus and Lysosomes, inhibition of SCV-lysosome fusion</td>
<td>(Liaquat et al., 2018; and Saxena et al., 2018)</td>
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<td>Ssa E</td>
<td>Acts as chaperone</td>
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<td>Ssc A</td>
<td>Acts as chaperone</td>
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<td></td>
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<td>Ssc F</td>
<td>SCV perinuclear migration, microtubule bundling and SIF formation</td>
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<td>Sse G</td>
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<td>Ttr genes</td>
<td>Tetrathionate respiration and outgrowth in the intestine</td>
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<td>SPI C</td>
<td>Disruption of vesicular transport</td>
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<td>SIF A</td>
<td>Salmonella containing vacuole membrane integrity</td>
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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 \textit{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 \textit{Salmonella} cytolethal distending toxin (S-CDT) (Miller \textit{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 \textit{S. enterica} serovar Typhi similar to that seen in other cells intoxicated with an exotoxin known as CDT. Similar to cells intoxicated with CDT, \textit{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 \textit{CdtB} gene located within the \textit{Salmonella} genomic islets (SPI). S-CDT is an A\textsubscript{2}B\textsubscript{5} toxin composed of a pentameric ring (Miller \textit{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 \textit{CdtB} gene is located, open reading frames (ORFs) 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 \textit{et al.}, 2008). It is also believed that the typhoid toxin evolved from the exotoxin ancestors; CDT and pertussis toxins (Galan, 2016).

![Complex of typhoidal toxin showing the 2 A subunits (blue and red) and 5 B subunits (green)](https://example.com/image.png)

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 \textit{et al.}, 2017).

An exotoxin which is heat labile known as \textit{Salmonella} enterotoxin (Stn) encoded by the \textit{stn} gene has been reportedly produced by \textit{S. enterica} serovar Typhi strains as detected by PCR and Southern blotting (Aphons \textit{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 \textit{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 \textit{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-κB, 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 α-1,4-2-deoxy-2-N-acetamido-2-deoxyglucose that is variably O acetylated at the C-3 position (Tran et al., 2010). The Vi capsule 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 SalmondIa 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-BA CDI) 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 Pstr98 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 Pstr98 had more severe disease with a higher rate of complication and mortality. Pstr98 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, fluoroquinolone, trimethoprim-sulfamethoxazole, 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<sub>TEM</sub>, bla<sub>SHV</sub>, bla<sub>CTX-M</sub>, sul, adaA<sub>2</sub>, 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 β-lactamases (ESBLs): TEM, SHV and CTX-M which is encoded by bla<sub>TEM</sub>, bl<sub>SHV</sub>, bl<sub>CTX-M</sub> genes respectively. Other genes coding for β-lactamases are bla<sub>OX</sub>, bl<sub>SE</sub>, bl<sub>SHV</sub>, bl<sub>TEM</sub>-β-lactamases are enzymes that inactivates an antimicrobial, by cleave the four-membered ring in β-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. β-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 β-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 (Riyaz 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 resistant 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 (dfrI, dfrN, dfrVII, dfrIX, dfrIII) 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 prominent (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 1 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 1 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 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 resistance...
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 \textit{S. enterica} serovar Typhi strong antimicrobial resistance. Drugs could be designed to maneuver the antibiotic resistance mechanisms employed by \textit{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 \textit{S. enterica} serovar Typhi and reducing the prevalence of MDR strains.

**References**


