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Extended Spectrum Beta Lactamase Producing Strains of *Salmonella species* - A Systematic Review

Adamu Ishaku Akyala[1,2,*], Selwa Alsam[1]

[1]Department of Biological Science, University of Essex Wivenhoe Park Colchester, UK
[2]Department of Biological Science, Microbiology Unit, Nasarawa State University, Keffi, Nigeria

Abstract *Salmonella* infections in humans can range from a self-limited gastro-enteritis usually associated with non-typhoidal *Salmonella* (N T S) to typhoidal fever with complications such as a fatal intestinal perforation . In Nigeria, like other developing countries, it is difficult to evaluate the situation of salmonellosis. This is mainly because of the very limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture. In addition, under reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis in some countries, including Nigeria. Salmonellosis causes significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact. Extended-spectrum β-lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. Typically, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have recently been described. The presence of ESBLs carries tremendous clinical significance. The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited. In common to all ESBL detection methods is the general principle that the activity of extended-spectrum cephalosporin's against ESBL-producing organisms will be enhanced by the presence of clavulanic acid. ESBLs represent an impressive example of the ability of Gram-negative bacteria to develop new antibiotic resistance mechanisms in the face of the introduction of new antimicrobial agents.

Keywords Extended, Spectrum, Beta, Lactamase, Salmonella, Strains

1. Introduction

Non-typhoidal *Salmonella* is one of the principal causes of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Tassios *et al*., 1997). Non-typhoidals fever, which is caused mainly by *Salmonella typhimurium and Salmonella enteritidis*, continues to be a major problem in developing countries. A recent study estimated that globally there are more than 22 million cases of typhoid fever each year with more than 200,000 deaths, however, the true magnitude is difficult to quantify because the clinical picture is confused with many other febrile illnesses and most typhoid endemic areas lack facilities to confirm the diagnosis (Crump *et al*., 2004).

Antibiotic treatment is not required for *Salmonella* gastroenteritis but is essential for enteric fever, invasive salmonellosis and in patients at risk of extra-intestinal disease. For many years chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole (cotrimoxazole) were the drugs of choice. In recent years, increasing resistance of *Salmonella* species to commonly used antimicrobials has become a matter of concern. Of particular concern are those strains that have acquired multiple drug resistance (MDR) against two or more therapeutic agents. Although fluoroquinolones, such as ciprofloxacin and ofloxacin, and extended spectrum cephalosporins, such as ceftriaxone and cefotaxime, have proved to be effective alternatives, resistance to these agents has emerged (Parry, 2003).

To track *Salmonella* infections and disrupt epidemic spread, many nations have established extensive surveillance systems. Typing to the strain level has been an important tool in surveillance and outbreak investigation of *Salmonella* infections. Most of these surveillance projects rely on traditional (phenotypic) methods like serotyping, phage and biotyping, which provide a limited means of distinguishing epidemic from endemic or sporadic isolates. Nowadays,

* Corresponding author:
akyalaisaac@yahoo.com (Adamu Ishaku Akyala)
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phenotypic methods are either replaced or complemented by more sensitive and discriminative molecular techniques. Typing schemes based on variation in particular DNA sequences are digital and the same results could be achieved wherever the test is performed. Sequence based typing schemes can also be considered as genetic classification schemes (Liebana, 2002; Winokur, 2003).

1.1. The Genus Salmonella

1.1.1. General Characteristics

Members of the genus *Salmonella* are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds, and insects. Salmonellae are Gram-negative, non- spore forming, facultative anaerobic bacilli, 2 to 3 by 0.4 to 0.6 μm in size. Like other members of the family *Enterobacteriaceae*, they produce acid on glucose fermentation; reduce nitrates to nitrite, and don't produce cytochrome oxidase (Farmer, 1995). Most organisms except *S. gallinarum–pullorum* are motile by Peritrichous flagella. The differential metabolism of sugars can be used to distinguish some *Salmonella* serotypes, e.g., most don't ferment lactose. *S. typhi* is the only organism that does not produce gas in sugar fermentation. *Salmonella* are non-capsulated except *S. typhi*, *S. Paratyphi* C and some strain of *S.* Dublin (WHO, 2003).

1.1.2. Antigenic Structures

The typical *Salmonella* are defined mainly by two sets of antigens, somatic (O) and Flagellar (H) that are readily demonstrable by serological reactions in the laboratory. In addition, other bacterial antigens are also available. These include: an exopolysaccharide (Vi, or virulence antigen), the mucus (M), and the fimbrial (F) antigens (Huckstep, 1962; Old, 1996).

a. O (Somatic) antigen

These somatic antigens represent the side-chains of repeating sugar units projecting outwards from the lipopolysaccharide layer on the surface of the bacterial cell wall. Typing the O antigen denotes the serogroup. Over 60 different O antigens have been recognized and they are designated by Arabic numerals. The O antigens are heat stable, being unaffected by heating for 2.5 hours at 100℃, and alcohol stable withstanding treatment in 96% ethanol at 37℃ (Lewis, 1998; Old, 1996).

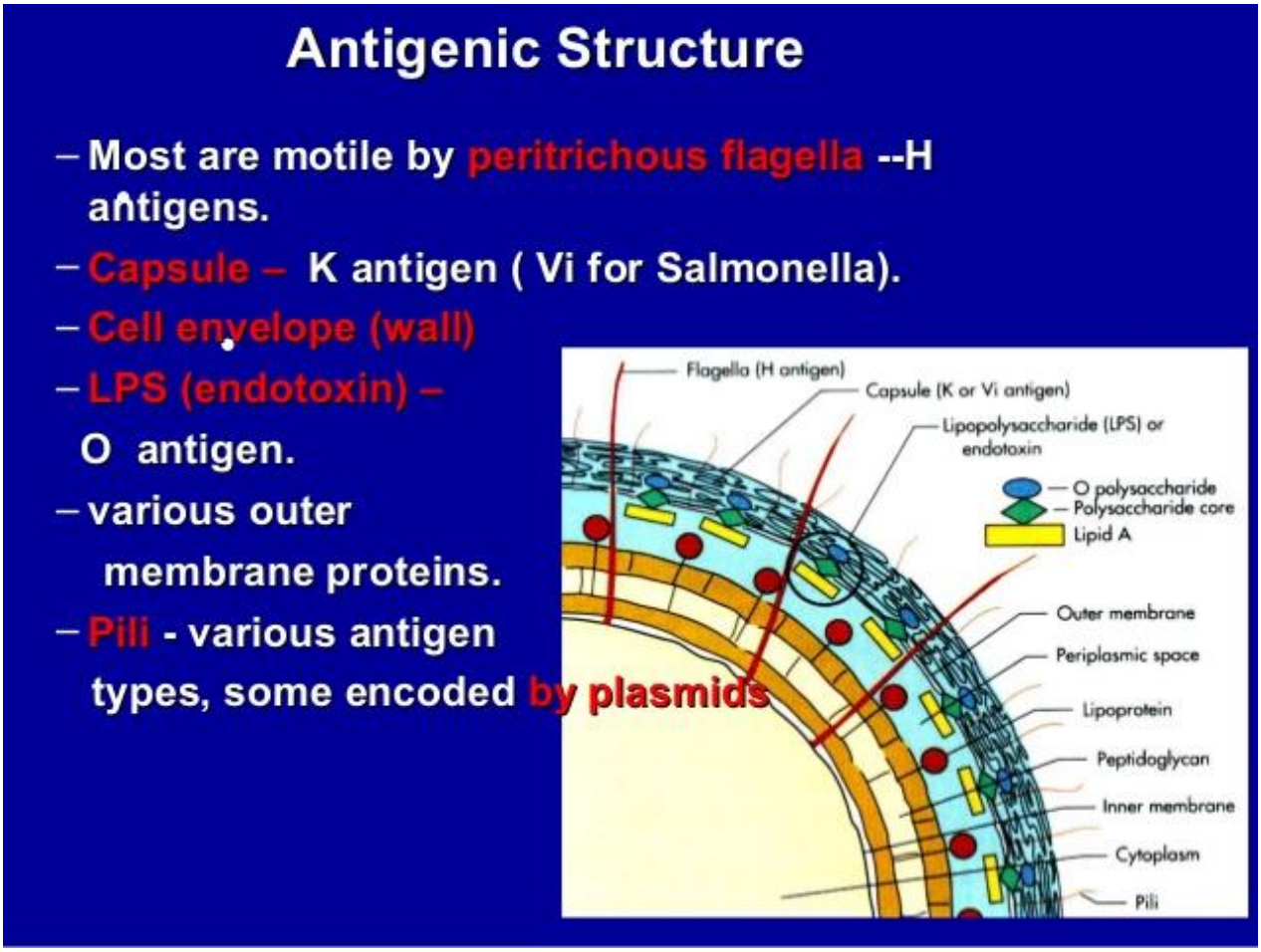


Figure 1. Antigenic structure of Salmonella species (Adapted from Huckstep, 1962)

b. H (Flagellar) antigen

Flagellar (H) antigens are formed from structural proteins, which make up the flagella that endow the organism with motility. They are heat labile. Heating at 60℃ and above detaches the flagella from the bacteria. *Salmonella* is unique among the *Enterobacteriaceae* in that it commonly has two distinct H antigens, the phase 1 (protein product of the *fli*C gene) and phase 2 (protein product of the *fljB* gene) flagellar antigens, that are coordinately regulated such that only one flagellar antigen is expressed at a time in a single cell (Smith and Selander, 1991). Some H antigens are composed of multiple antigens, termed factors; for example, H: e,n,x is the designation for a flagellar antigen that consists of three separate factors, e, n, and x, that occur together in one flagellum. These factors represent different epitopes on the flagellar protein.

The 114 H antigens are composed of combinations of 99 distinct antigenic factors. Flagellar antigens that are immunologically related are known as complexes. For example, the G complex includes all flagellar antigen types that contain antigenic factor g (e.g., g,m; f,g; g,z_{51}), plus flagellar antigen m,t. Flagellar antigen types that include antigen H:z_4 are considered the Z4 complex (Smith and Selander, 1991). The flagellar antigens of phase I are labeled with lower cases letters (a to z and z_1 z_2 z_3 etc.) and phase 2 with a mixture of lower case letters and Arabic numerals.

The antigenic structure of any *Salmonella* is expressed as an antigenic formula which has three parts, describing the O antigens, the phase I and II flagellar antigens respectively (Lewis, 1998). Phase II used to be termed "the group" or non-specific antigens because numerous serotypes of salmonellae share the same antigens when it is this phase. The presumptive identification of the serovars, therefore mainly depends on the identification of the H antigens in phase I, which are relatively 'specific' (Old, 1996). As an example, the antigenic formula for the serovar *Salmonella typhimurium* – **1, 4, 5, 12 : i : 1, 2** were 1, 4, 5 and 12 are O antigens, i is phase 1 H antigen and 1 and 2 are phase 2 H antigens (Kauffmann, 1950).

2. Classification of *Salmonella*

The classification of *Salmonella* is complex because the organisms are a continuum rather than a defined species. The current classification of the genus *Salmonella* is based on DNA-DNA hybridization studies (Crosa *et al*., 1973). This work suggested that the genus *Salmonella* is divided into two species known as *Salmonella bongori* and *Salmonella enterica. S. enterica* can be further divided into 6 distinct subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*), based on different biochemical profiles, in addition to genetic relatedness (Brenner *et al*., 2000). The majority of the 2500 known serovars that causes disease in warm-blooded animals are found in *Salmonella enterica* subspecies *enterica* as shown in Figure 2 (Langridge *et al*., 2008).

The Kauffmann-White classification scheme classified the *Salmonella* into different O groups and serovars based on the expression of somatic lipopolysaccharide 'O' antigen and flagellar 'H' antigens respectively (Kauffmann, 1950). The O group's first defined were designated by capital letters A to Z and those discovered later by the number (51-67) of the characteristics O antigen (Old, 1996). It is now considered more correct to designate each O group by its characteristic O factor, i.e. to abandon the letters A-Z used to designate early O groups. Hence, O groups become: O2 (A), O4 (B), O7 (C_1), O8 (C_2), O9, 12(D_1) etc. (Old, 1996). Groups O2 to 03, 10 (A-E) contain nearly all the salmonellae that are important pathogens in man and animals (Brenner *et al*., 2000). Newly described serovars are listed in regular updates of the Kauffman and White scheme.

2.1. Salmonella Nomenclature

Salmonella nomenclature is complex, since different scientists use different systems to refer and communicate about this genus. Some individuals prefer Kauffman's initial 'one serovar-one species' concept, while others favor schemes based on clinical presentation, biochemical characteristics or genetic relatedness (Langridge et al.2008)).

According to the Center for Disease and Control (CDC) system, the genus *Salmonella* contains two species, each of which contains multiple serotypes. CDC uses a name for serovars in subspecies I (for example. serovar *Salmonella typhimurium*) and uses antigenic formulas for unnamed serotypes (Brenner *et al*., 2000). At first citation of a serotype the genus name is given followed by the word 'serovar' or the abbreviation 'ser' and then the serotype. For example, *Salmonella* serotype or ser *Salmonella typhimurium* subsequently the name may be written with the genus followed directly by the serotype (for example, *Salmonella typhimurium* (Popoff *et al*., 1998).

2.2. Genome of Salmonella

Because of the presence of large number of serovars in *Salmonella*, the genome sequence projects concentrated on serovars that are either of importance to human disease or a representative of a particular branch of the *Salmonella* (Mastroeni, 2006). Genome information can be used to gain insights into the evolution of the *Salmonella* genus, to identify stable regions conserved between different *Salmonella* species and serovars, and to identify regions that appear to be specific for individual serovars (Mastroeni, 2006).

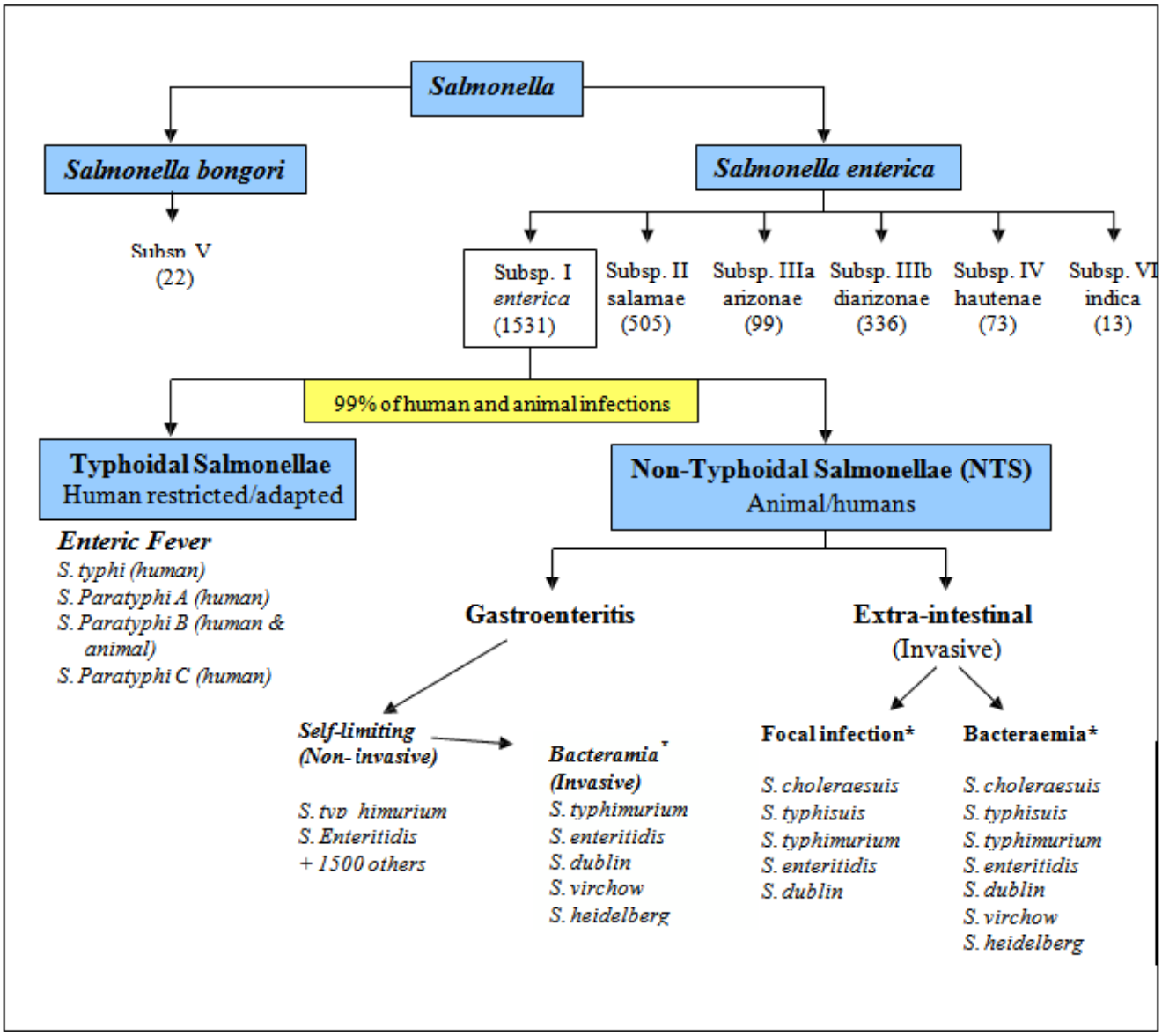
DNA sequence comparison between the genome of *S.* typhimurium LT2 and *S. typhimurium* CT8 show a median homology of 98% (McClelland *et al*., 2001). Comparison of the genes required for DNA replication, transcription, translation and central metabolism ('housekeeping' genes) of the *Salmonella* serovars indicates that they are extremely similar at the DNA level. Pair wise comparisons between any of the sequenced *Salmonella* genomes indicate that the similarity between housekeeping genes ranges from 97.6%

to 99.5% at the DNA level (Edwards *et al*., 2002).

The complete genome sequence was determined for a multidrug-resistant strain of *S. enterica* serotype *Salmonella typhimurium* (CT18). The CT18 genome harbors 4,809,037 base pairs with an estimated 4599 coding sequences. Significant homology has been seen among genomes of *S.* Typhi-CT18, *Salmonella typhimurium* LT2 (McClelland *et al*., 2001) and *E. coli* K12 (Blattner *et al*., 1997) genomes, indicating a common evolutionary origin.

Salmonella typhimurium is also a member of sub-species I and is the leading cause of gastroenteritis in humans and unlike *S. typhi* can infect mice and cause a typhoid-like disease. The genome size of *Salmonella typhimurium* LT2 is 4,857 Kbp (McClelland *et al*., 2001) and sequence comparison between these two organisms revealed 89% of *S. Salmonella typhimurium* LT2 CDSs were homologous to *S. typhi* CT18 at the nucleotide level (McClelland *et al*., 2001). Although genetically similar to the *E. coli* K12 genome, both *S.*typhimurium and *Salmonella typhimurium* have acquired large regions of extraneous DNA by horizontal transfer known as pathogenicity islands, which offer a selective pathogenic advantage to the organisms (Marcus *et al*., 2000).

Salmonella enterica strains contain plasmids which vary in size from 2 to more than 200 kb. There are different types of plasmids in *Salmonella* and the best described groups of plasmids are the virulence plasmids (50–100 kb in size) present in serovars enteritidis, *Salmonella typhimurium* Dublin, Cholerae-suis, Gallinarum, Pullorum and Abortus-ovis. The virulence plasmids have a common 8kb DNA region encoding the *spv (Salmonella* plasmid virulence) gene involved in intracellular macrophage survival of *Salmonella.*



Numbers in brackets indicate the total number of serotypes included in each subspecies.

* Common serotypes are listed but other serotypes may cause bacteraemia or focal infection; subsp., subspecies.

Figure 2. Classification of the genus *Salmonella*. (Adapted from Langridge *et al*. 2008)

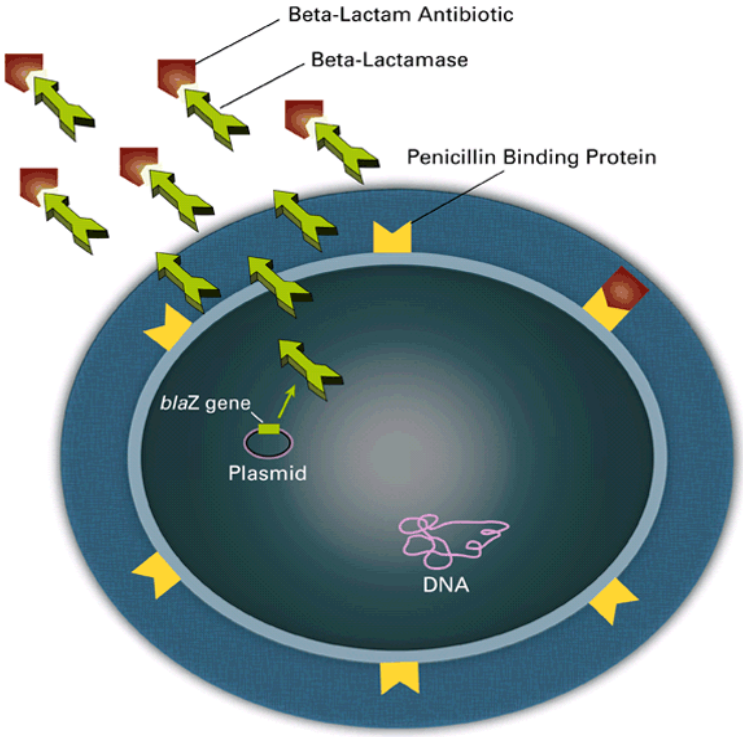


Figure 3. Schematic representation of plasmid-mediated horizontal transfer of antibiotic resistance

(Gulig *et al*., 1993). Depending on serovar these plasmids code for additional virulence- associated genes such as *rck* (resistance to complement killing), *pef* (plasmid encoded fimbriae), *srgA* (SdiA-regulated gene, putative disulphide bond oxidoreductase) or *mig-5* (macrophage-inducible) gene coding for putative carbonic anhydrase) (Rychlik *et al*., 2006).

Another group of high molecular weight plasmids are responsible for antibiotic resistance. The antibiotic resistance genes are often located within transposons which can transpose from plasmids to chromosome, and vice versa (Figure 3). Resistance genes can be also found in a form of gene cassettes incorporated into integrons (Hall and Collis, 1995). Small ColE1-type plasmids (pC) of 3-5.6kb have been found in *S. enterica serovar* Enteritidis and one of these plasmids carries an active restriction modification system, which could explain the high resistance of pC-carrying *S. enterica* serovar Enteritidis strains to phage infections (Gregorova *et al*., 2002).

Resistance genes are indicated by shadowy boxes. Resistance genes are often found located within transposons and can be transposed into the bacterial chromosome or incorporated within plasmids, indicated by white circles. Resistance genes can also be located as gene cassettes within integrons. A gene cassette consists of one complete open reading frame followed by a recombination site (black box). Gene cassettes exist free as covalently closed circular molecules generated by the integrase and the circular intermediate participates in the integrase-mediated process of insertion. As many as seven different gene cassettes have been described within a single integron (Adapted from Carattoli, 2003).

3. *Salmonellosis* (The Disease)

3.1. Pathogenesis

Salmonellosis in the human host is generally associated with *Salmonella enterica* subspecies *enterica* (also termed subspecies I) and acute infections can present in one of four ways: enteric fever, gastro-enteritis, bacteraemia, and extraintestinal (EI) focal infection. As with other infectious diseases the course and outcome of the infection are dependent upon a variety of factors including inoculating dose, immune status of the host and genetic background of both host and infecting organism (Cammie and Miller, 2000).

Broadly speaking the *Salmonella enterica* from human infections can be subdivided in to two groups: the enteric fever (typhoidal) group and non-typhoidal *Salmonella* (NTS), which typically cause gastro-enteritis but can cause invasive disease under certain conditions. There are five serotypes of *Salmonella* associated with enteric fever: *Salmonella enterica* subspecies *enterica* serovar *Salmonella typhimurium*, *S.* Paratyphi A, *S.* paratyphi B, *S. paratyphi C* and *S.* Sendai. *S. typhi* forms a genetically homogenous group as do and *S. paratyphi A* and Sendai together, whereas *S. paratyphi B and C* are heterogeneous (Selander *et al*., 1990).

All *Salmonella* infections begin with the ingestion of organisms in contaminated food or water. After leaving the stomach, *Salmonella* must traverse the mucosal layer

overlaying the epithelium of the small intestine. After crossing the mucosal layer overlaying the intestinal epithelium, *Salmonella* interacts with both enterocytes and Microfolds cells (M cells) (Francis *et al*., 1992). The organisms are rapidly internalized and transported into submucosal lymphoid tissue where they may enter into systemic circulation. *Salmonella* have also the ability to induce non phagocytic epithelial cells by a process known as bacterial mediated endocytosis. This process involves the formation of large membrane ruffles around the organism and cytoskeleton rearrangement (Francis *et al*., 1992). *Salmonella* is then internalized within bound vacuoles through which organisms' trancytose from the apical to the basolateral surface (Rathman *et al*., 1997). Once it crosses the intestinal epithelium, *Salmonella* serotypes that cause systemic infections enter macrophages, and migration of infected macrophages to other organs of reticulo endothelial systems probably facilitates the dissemination of bacteria in the host (Figure 4).

Gastroenteritis due to NTS may persist with fever, nausea, vomiting, abdominal pain and symptoms may continue for over a week. In contrast, the early symptoms of enteric fever are often vague, and may include a dry cough, severe headache, anorexia, fever and a tendency to constipation rather than diarrhoea (Parry *et al*., 2002). If enteric fever is not treated on time, serious complication like hemorrhage from ulcers can occur during the third week of illness or perforation of the Peyer's patches (PP) can cause generalized peritonitis and septicemias; these are the commonest cause of death in typhoid fever. With the introduction of early and appropriate antibiotic therapy, the average case fatality rates for typhoid are less than 1% (Everest *et al*., 2001).

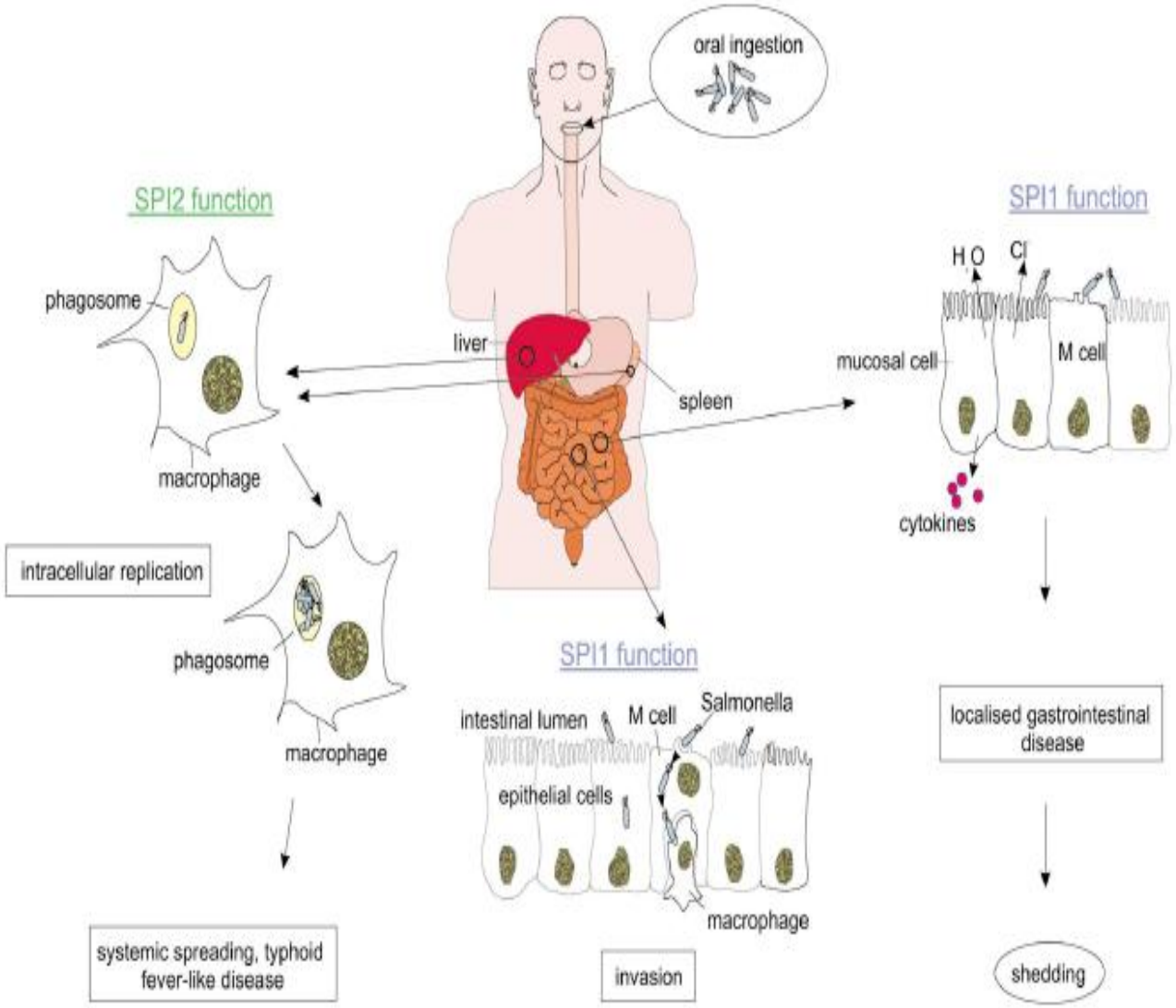


Figure 4. Schematic representation of host–pathogen interactions during pathogenesis of *Salmonella* infections. *Salmonella* Pathogenicity Island (SPI1) function is required for the initial stages of salmonellosis, i.e. the entry of *Salmonella* into non- phagocytic cells by triggering invasion and the penetration of the gastrointestinal epithelium. Furthermore, SPI1 function is required for the onset of diarrhoeal symptoms during localized gastrointestinal infections. The function of SPI2 is required for later stages of the infection, i.e. systemic spread and the colonization of host organs. The role of SPI2 for survival and replication in host phagocytes appears to be essential for this phase of pathogenesis (Adapted from Hansen-Wester and Hensel, 2001)

3.2. Virulence Factors

The outcome of a *Salmonella* infection is determined by the status of the host and status of the bacterium (Figure 4). The status of the bacterium is determined by the so- called virulence factors (van Asten and van Dijk, 2005).

a. Salmonella Pathogenisity Islands (SPIs)

The majority of virulence genes of *Salmonella* are clustered in regions distributed over the chromosome called *Salmonella* pathogenicity islands (McClelland *et al*., 2001). The SPIs are of major importance for the virulence of *S. enterica*. Hallmarks of *Salmonella* virulence, such as cell invasion, intracellular survival and the production of Vi antigens capsule are encoded by SPIs. Until recently more than 10 SPIs have been identified on the *Salmonella* chromosome, but SPI-1 and SPI-2 is the central for pathogenesis of *Salmonella* infections (Hansen-Wester and Hensel, 2001).

All types of *S. enterica* have two large clusters of genes known as *Salmonella* Pathogenicity Island 1 and 2. *Salmonella* Pathogenicity Island 1 encodes genes necessary for invasion of intestinal epithelial cells and induction of intestinal secretory and inflammatory response (Galyov *et al*., 1997). *Salmonella* lacking a functional SPI-1 Type III secretion system are unable to invade epithelia cells and induce cytokine synthesis (Hobbie *et al*., 1997). During invasion of the gut, the SPI-1 encoded SipB protein triggers the activation of intracellular Caspase-1 within resident macrophages that induces apoptosis in the infected macrophages resulting in escape of *Salmonella* from these cells (Hersh *et al*., 1999). SPI-1 also encodes an effector protein SopB which is an inositol phosphate phosphatase and its enzymatic activity results in activation of chloride channel in the membrane of epithelial target cells leading to the secretion of chloride and loss of fluid into the intestinal lumen (Norris *et al*., 1998).

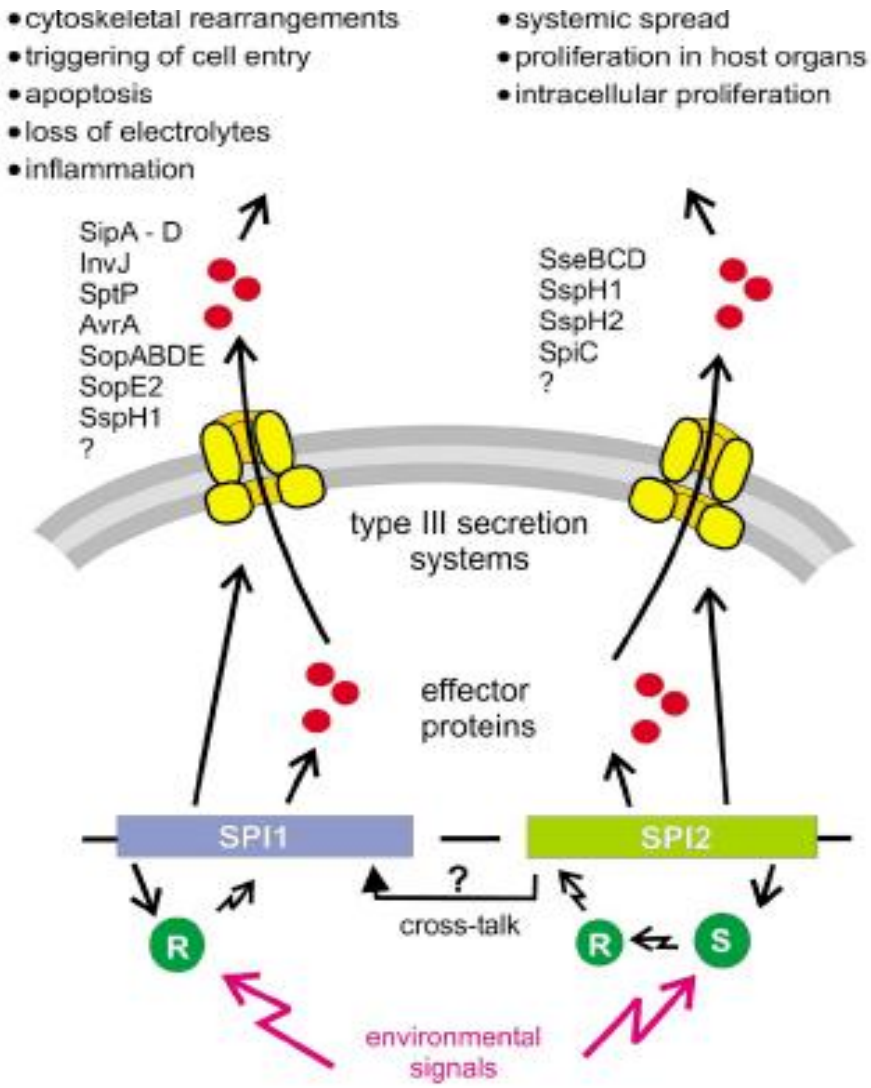


Figure 5. Model for the functions of two TTSSs involved in the interaction of *Salmonella* spp. with host cells. SPI1 and SPI2 both encode TTSSs and each TTSS has a distinct set of secreted substrate proteins. Furthermore, both SPIs encode specific regulators (R, DNA-binding regulator; S, sensor) that control gene expression. The function of each system is required for different forms of interaction with host cells at different stages of pathogenesis of salmonellosis (Adapted from Hansen-Wester and Hensel, 2001)

Salmonella Pathogenicity Island 2 encodes genes essential for intracellular replication and necessary for establishment of systemic infection beyond the intestinal epithelium (Hensel, 2006). The function of the SPI-2 encoded Type III secretion system is required to protect the pathogens within the *Salmonella* containing vacuole (SCV) against the effectors functions of innate immunity. It has been reported that SPI-2 prevents co- localization of the phagocyte oxidase (Vazquez-Torres *et al*., 2000) and the inducible nitric oxide synthtases to the SCV (Chakravortty *et al*., 2002). As a consequence, intracellular *Salmonella* are protected against damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Boonmar *et al*., 1998) and against a potent antimicrobial activity of peroxynitrite, which is generated by reaction of reactive nitrogen species and reactive oxygen species (Chakravortty *et al*., 2002). SPI-2 genes enabled *S. enterica* lineages to establish a new niche as an intracellular pathogen in the intestinal mucosal and systemic tissue.

b. Type III secretion systems

Central to the pathogenesis of *S. enterica* is the function of specialized protein secretion systems, known as Type III secretion system (TTSS). TTSS are specialized virulence devices that have evolved indirect translocation of bacterial virulence proteins into the host cell cytoplasm. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope (Galan, 1998). So far the presence of two SPIs (SPI-1 and SPI-2) each encoding a TTSS, have been described for *Salmonella* species and may reflect the flexibility of this highly successful pathogen in causing different forms of diseases. SPI-1 is not present in *E. coli* isolates, this suggest that the acquisition of SPI-1 by *Salmonella* was a fundamental step in the divergence of these two genera (Hansen-Wester and Hensel, 2001).

c. Regulatory proteins, toxins, plasmids and Vi antigens

Regulatory proteins that control the synthesis of multiple proteins at the level of gene transcription are also essential to *Salmonella* pathogenesis. The best studied example is PhoP/PhoQ, which regulates genes important for survival within macrophages, resistance to cationic antimicrobial proteins and acid pH, and invasion of epithelial cells (Behlau and Miller, 1993). PhoP/PhoQ regulated genes encode an acid phosphatase, cation transporters, outer membrane proteins, and genes important for the modification of lipopolysaccharide; these modifications promote resistance to antimicrobial cationic peptides and alter the ability of lipid A to stimulate tumor necrosis factor-α secretion by macrophage (Guo *et al*., 1997).

Other regulatory genes implicated in pathogenesis include *ompR/envZ*, the regulator of porin gene transcription; *katF*, an alternative bacterial sigma-factor that regulates catalase production; and *ssrAB*, which regulates genes in SPI-2 that are important for systemic pathogenesis (Lindberg, 1980). Non-typhoidal *Salmonella* also carry a variety of virulence plasmids which might play a role in multiplication inside the cell, destabilizing the cytoskeleton of the eukaryotic cell and also might be involved in resistance of *Salmonella* species to the bacteriolytic activity of serum. Enterotoxin may also play a role in *Salmonella* gastroenteritis. An enterotoxin antigenically similar to Cholera toxin also has been identified (Aguero *et al*., 1991). Flagella phase variation that is exploited by the majority of flagellated *Salmonella* might be related to escaping the host defense system (van Asten and van Dijk, 2005).

The Vi antigen of *S.* Typhi prevents antibody mediated opsonization, increases resistance to peroxide, and confers resistance to complement activation by the alternative pathway and to complement mediated lysis (Looney and Steigbigel, 1986).

3.3. Immunity

The immune response in *Salmonella* includes innate and adaptive immunity. The intestinal epithelium, neutrophil, macrophage, dendritic cell, NK cell, and γδT cells take important part in innate immunity and antigen specific T and B cells take part in the adaptive immunity (Mizuno, 2004).

The following are some early defense mechanisms in the gut: a) gastric acid may directly kill the bacteria or activate the proteolytic activity of pepsin which is required for the cleavage of Histone 2A, into antibacterial peptide (Mastroeni, 2006); b) phagocyte and innate immunity: usually phagocytic cells control the growth of *S. enterica* in the first few days of the infection using reactive oxygen species generated via the phagocyte NADPH- oxidase (Mastroeni *et al*., 2000), while RNS, that are produced following the activation of the inducible nitric oxide synthase, play a role in resistance in the later stage of infection of *S. enterica* (Mastroeni *et al*., 2000); c) Cytokines are key regulators of the host responses in intracellular pathogenesis and various bacterial products from *Salmonella* are potent inducers of cytokine expression by immune cells (Lalmanach and Lantier, 1999); d) antibody response to *S. enterica* infections induce early IgM antibody responses followed by IgG and IgA production (Mastroeni, 2006).

A large number of antigens including LPS determinants (O-polysaccharide and core regions), Vi antigen, porins, outer membrane proteins, lipoproteins, heat shock proteins, flagella and fimbriae are recognized by *Salmonella*-specific antibodies (Mastroeni, 2002); e), T cell response: *S. enterica* infections induce proliferation of CD4+ T cells which plays a pivotal role in activation of macrophage (Mastroeni, 2002). CD8+ T cells is also involved in producing IFN-γ and lysing target cells infected with *S. enterica* (Salerno-Goncalves *et al*., 2003). Suppression of the growth of *S. enterica* is followed by the elimination of the bacteria from the tissue. If the bacteria are not cleared, a late resurgence of bacterial growth can occur (relapse), or a chronic carrier state can develop, which can be a serious problem since it constitutes a reservoir of infection (Mastroeni, 2006).

4. Mechanism of Drug Resistant

Efforts aimed at identifying new antibiotics were once a top research and development priority among pharmaceutical companies. The potent broad spectrum drugs that emerged from these endeavors provided extraordinary clinical efficacy. Success, however, has been compromised. We are now faced with a long list of microbes that have found ways to circumvent different structural classes of drugs and are no longer susceptible to most, if not all, therapeutic regimens (Alekshun and Levy, 2007).

Resistance to various classes of antimicrobial agents has been encountered in many bacteria of medical and veterinary relevance. Over the years, various studies have reported the presence of genes and mutations conferring resistance to antimicrobial agents in zoonotic bacteria such as *Salmonella* (Michael *et al*., 2006).

There are three major mechanisms by which bacteria have become resistant to antimicrobial agents: enzymatic inactivation; reduced intracellular accumulation of the antimicrobials; protection, alteration or replacement of the cellular target sites (Schwarz and Chaslus- Dancla, 2001).

a. Chloramphenicol

Chloramphenicol (CAF) is a broad-spectrum bacteriostatic agent and the antibacterial action of chloramphenicol is mediated by inhibiting protein synthesis after binding to the 50S subunit of the bacterial ribosome and thus preventing the transfer of the new amino acid from its tRNA to the growing peptide chain. This binding is achieved by molecular mimicry of the peptidyl adenyl terminus of the tRNA molecule (Schwarz *et al*., 2004). The first and still most frequently encountered mechanism of bacterial resistance to CAF is enzymatic inactivation by acetylation of the drug via different types of chloramphenicol acetyltransferases (CATs) (Murray and Shaw, 1997). However, there are also reports on other mechanisms of CAF resistance, such as efflux systems, inactivation by phosphotransferases, mutations of the target site and permeability barriers (Murray and Shaw, 1997).There are two defined types of CATs which distinctly differ in their structure: CatA and CatB enzymes. In *Salmonella,* enzymatic inactivation by type A or type B chloramphenicol acetyltransferases (Cat) as well as the export of chloramphenicol or chloramphenicol/florfenicol by specific efflux proteins is the dominant resistance mechanisms (Michael *et al*., 2006). Two different types of CatA proteins, encoded by the genes *catA1* and *catA2*, have so far been detected in *Salmonella* isolates. While the Tn9- borne resistance gene catA1 has been found in several serovars, including *Salmonella typhimurium* (Chen *et al*., 2004), the gene *catA2* was detected on a multiresistance plasmid from *Salmonella typhimurium*, and *Salmonella typhimurium* (Randall *et al*., 2004)).

Three different types of *catB* genes, *catB2*, *catB3* and *catB8*, are known to occur in *Salmonella*. All these *catB* genes are located on gene cassettes and have mainly been identified in class 1 multi-resistance integrons in *Salmonella typhimurium* (Nogrady *et al*., 2005). The chloramphenicol exporter gene *cmlA* is also a cassette borne gene which has been found in plasmid-located class 1 integrons in *Salmonella typhimurium* (Nogrady *et al*., 2005).

b. Co-trimoxazole

A combination of trimethoprim and sulphamethoxazole, known as co-trimoxazole has been successfully used to treat *Salmonella* infections. Since these two antimicrobials inhibit sequential stages in tetrahydrofolic acid (THFA) synthesis it was believed that administration of a combination therapy would have a selective advantage over a single administration (Nogrady *et al*., 2005).

Sulfonamides and trimethoprim block different enzymatic steps in tetrahydrofolate biosynthesis (Figure 9). Sulfonamides are structural analogs of *p*-aminobenzoic acid and competitively inhibit the enzyme dihydropteroic acid synthase (DHPS) while trimethoprim competitively inhibits the enzyme dihydrofolate reductase (DHFR) (Sköld, 2001).

Chromosomal, plasmid and transposon mediated resistance have all been reported for this antimicrobial. Chromosomal mutations involve the over production of the dihydrofolate reductase (DHFR), which leads to trimethoprim resistance involving the need for a higher inhibitor concentration of drug inside the cell to decrease the residual enzyme activity. The commonest mechanism of trimethoprim resistance is associated with the production of an additional non-susceptible form of DHFR encoded by genes located on self-transmissible or mobile plasmids and transposons (Huang *et al*., 2004).

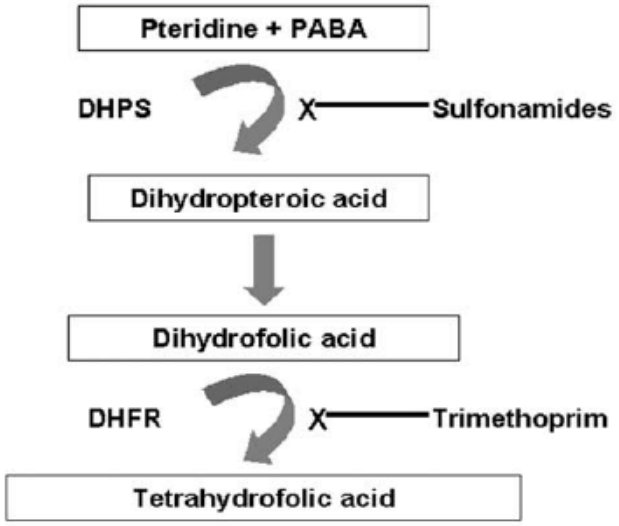


Figure 1. Inhibition of folate synthesis by sulfonamides and trimethoprim. PABA, paraaminobenzoic acid; DHPS, dihydropteroate synthase; DHFR, dihydrofolate reductase (Adapted from Huang *et al*., 2004) So far, more than 30 different trimethoprim resistance mediating dihydrofolate reductase (*dfr)* genes have been identified. These are subdivided on the basis of their structure into two major types 1 and 2, which nowadays are referred to as *dfrA* and *dfrB*. While *dfrB* genes have not yet been identified in *Salmonella*, a total of 13 different *dfrA* genes-most of which are cassette-borne genes located in class 1 or class 2 integrons have been sequenced from various *Salmonella enterica* serovars (Michael *et al*., 2006)

c. Fluoroquinolones

Fluoroquinolones (FQ) represent the second, third and fourth generation of generation of quinolone development. The first generation of this group of compounds is represented by agents such as nalidixic acid and pipemidic acid and are characterized by limited activity against Gram-negative bacteria (Bager and Helmuth, 2001). The second-generation quinolones (namely ciprofloxin, norfloxacin, ofloxacin) have increased potency and antibacterial spectrum by modifying the original two-ring quinolone nucleus with different side chain substitutions and introducing fluorine at the 6th position. Their use now accounts for about 11% of overall prescriptions of antimicrobials in human medicine and one of them, ciprofloxacin, is the most used antibiotic in the world (Acar and Goldstein, 1997).

Fluoroquinolones mechanism of action in *Salmonella* include inhibition of tertiary super coiling of bacterial DNA, primarily by inhibiting the action of DNA gyrase, a Type II topoisomerase, which consists of two GyrA and two GyrB subunits encoded by *gyrA* and *gyrB*, respectively (Figure 7) (Anderson, 2007). A single point mutation in *gyrA* between amino acids 67 and 106 (known as the quinolone resistance-determining region or QRDR) can give rise to nalidixic acid (a first-generation quinolone) resistance among isolates of *Salmonella*. This resistance is usually accompanied by a reduction in the susceptibility (MIC 0.125-1.0mg/L) of these isolates to ciprofloxacin (Murray *et al*., 2005).

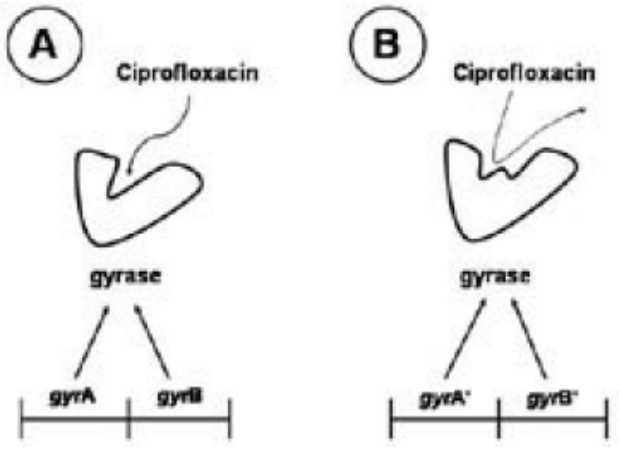


Figure 7. Mechanism of ciprofloxacin resistance. (A) Ciprofloxacin interacts with gyrase, inhibiting its enzymatic activity. (B) A mutation in either of the genes, gyrA or gyrB, can change the conformational structure of gyrase, and reduce the binding affinity of the enzyme for ciprofloxacin (Adapted from Andrson, 2007)

A study of >1000 stored *Salmonella* isolates from Finland has confirmed that show that resistance to nalidixic acid by means of disk diffusion is a sensitive and specific way of screening *Salmonella* isolates for reduced susceptibility to fluoroquinolones

(Hakanen *et al*., 1999). Resistance to nalidixic acid appears to be a predictor of clinical "quinolone hyporesponsiveness," and it is a harbinger of bona fide resistance to the clinically useful fluoroquinolones.

Mutation in *gry*B, which encodes the B subunit of gyrase, was identified at a much lower frequency than *gyr*A mutations. As in *E. coli* and other Gram-negative bacteria, topoisomerase IV, whose *parC* and *parE* genes are, respectively, homologous to *gyrA* and *gyrB*, is considered a secondary target for quinolones in *Salmonella*. This means that mutational modifications of this enzyme are expected to occur only in strains which already possess a mutated gyrase. Quinolone-resistance mutations in parC generally occur at codons Ser80 or less frequently at codon Glu84, which are homologous, respectively, to the Ser83 and Asp87 codons of gyrase (Casin *et al*., 2003). It is assumed therefore that the mode of action of quinolones against both enzymes is similar. In Gram negative bacteria, for many fluoroquinolones, DNA gyrase is the primary target and topoisomerase IV may be a secondary target associated with high level resistance. However, for many fluoroquinolones in Gram positive bacteria, such as *Staphylococcus aureus*, topoisomerase IV is the primary target (Wain, 2000).

Fluoroquinolones resistance mechanisms also include decreased accumulation due to active efflux and possibly decreased outer membrane permeability. Very recently, the presence of a plasmid-borne *qnr*-like gene conferring low-level quinolone resistance has been reported in clinical isolates of *S. enterica* serovar Enteritidis (Cheung *et al*., 2005). Thus, FQ resistance as observed in *Salmonella* isolates is the endpoint result of the accumulation of several, sometimes cooperating, and biochemical mechanisms, themselves resulting from various genetic events.

d. β-lactam antibiotics

The mode of action of beta lactam antibiotics is via penicillin binding proteins (PBPs) and inactivation of transpeptidases. The final stage in the synthesis of bacterial cell wall peptidoglycan is the transpeptidation of the free peptide strands of the cell wall peptide-glycan sub-units. This is achieved by acylation of the terminal amine group of one peptide chain onto the D-alanine residue of the next peptide chain (Wain, 2000).

Resistance to β-lactam antibiotics is mainly mediated by a large number of β- lactamases which differ in their abilities to hydrolyse the various beta-lactam antibiotics (Livermore, 1995). These enzymes can bind β-lactam molecules thus protecting the penicillin binding proteins in bacterial cell walls. Once bound, the acylation of a serine residue at the active site of the β-lactamase results in cleavage of the β-lactam ring of the antibiotic and regeneration of the β-lactamase. Other resistance mechanisms include the acquisition of penicillin binding proteins with reduced affinity to β-lactams, mutations in the PBPs (Schwarz *et al*., 2004). Reduced β-lactam uptake due to alterations in the outer membrane of gram negative bacteria or export by multi-drug transporters have been also reported (Paulsen *et al*., 1996).

Resistance against β-lactam antibiotics (penicillin and cephalosporins) in *Salmonella* is mainly mediated by

β-lactamase enzymes, which inactivate the antibiotics (Bush *et al*., 1995). The β-lactamases so far detected in *Salmonella* constitute a diverse group of enzymes encoded by a considerable number of genes. At least 10 different subgroups of b-lactamase genes (*bla*) coding for TEM-, SHV-, PSE-, OXA-, PER-, CTX-M-, CMY-, ACC-, DHA-, or KPC-type β-lactamases have been identified (Michael *et al*., 200=

e. Ampicillin

Bacterial resistance to ampicillin is mediated most commonly by a β-lactamase enzyme and the main family of β-lactamases responsible for ampicillin resistance is the temoxicillinase (TEM) β-lactamases. The gene for this enzyme has been shown to be present in resistant isolates of *Salmonella typhimurium* from Mexico and South East Asia (Taylor and Brose, 1985).

f. Cephalosporins

The cephalosporin nucleus is synthesized with a β-lactam ring attached to a six membered dihydrothiazine ring and based on cephalosporin C. Their mode of action is similar to the pencillins and unlike the penicillin nucleus; the cephalosporin nucleus is much more resistant to β-lactamase.

Cephalosporins are classified by four groups or subdivided into four generations based on the spectrum of their activity (Livermoore and Williams, 1996). First-generation cephalosporins (cefalotin, cefaloridin, cephalexin, cephapirin, cefazolin, cefadroxil, cephradine, and others) possess high biological activity with respect to *Staphylococci, Streptococci, Pneumococci*, and many types of enteric bacteria. Second-generation cephalosporins (cefuroxime, cefamandole, cefoxitin, cefotetan, cefaclor, and others) are characterized by high activity with respect to Gram-positive microorganisms that are resistant to β-lactamase action. They do not have a noticeable effect on enterococci (Parry *et al*., 2002).

Third-generation cephalosporins (cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefoperazone, and many others) differ in the highly antimicrobial activity against *Enterobacteriaceae* and are effective in treating typhoid fever. The fourth generation cephalosporin (Cefepime) is effective against Gram-positive (including methicillin- susceptible *S. aureus*, α hemolytic streptococci, and some coagulase negative staphylococci) and Gram-negative organisms, including *P. aeruginosa* (Nathisuwan *et al*., 2001).

Resistance to the broad spectrum cephalosporin is mainly due to the production of an enzyme called the extended spectrum β –lactamase (ESBL). The ESBL enzymes are plasmid - mediated enzymes capable of hydrolyzing and inactivating a wide variety of β lactams, including third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-I. All of these β-lactamase enzymes are commonly found in the *Enterobacteriaceae* family. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs (Paterson and Bonomo, 2005). These enzymes mediate resistance to cefotaxime, ceftazidime and other broad spectrum cephalosporins and to monobactams such as aztreonam, but have no detectable activity against cephamycins and imipenem. Because, of their greatly extended substrate range these enzymes were called extended spectrum β- lactamases (Cheung *et al*., 2005).

Widespread fluoroquinolone use in children has been discouraged because of the potential adverse effects on cartilage development. Therefore, extended-spectrum cephalosporins (especially cefotaxime or ceftriaxone) are the mainstay of treatment of serious infections due to *Salmonella* species in children. The production of ESBLs or AmpC β-lactamases consequently has considerable implications for clinical microbiology laboratories and physicians in areas in which infections with *Salmonella* species are common (Kruger *et al*., 2004).

Although reports of ESBLs associated with *Salmonella* spp. are relatively rare compared to those for other species in the family *Enterobacteriaceae*, the number of reported cases in this organism has been increasing in recent years. *Salmonella* have been found to express a wide variety of ESBL types, including TEM, SHV, PER, OXA, and CTX-M enzymes (Winokur *et al*., 2001). The genes encoding these extended-spectrum cephalosporinases are carried by conjugative plasmids, transposons, or integrons. These mobile genetic elements could spread horizontally between enteric organisms. Thus, not only can antimicrobial resistance in salmonellae emerge because of the selection pressure derived from inappropriate antimicrobial use in food animals, but drug-susceptible salmonellae can also become resistant via the in vivo acquisition of drug resistance plasmids from other enteric pathogens in the intestinal tract of patients (Su *et al*., 2003). ESBLs are typically encoded on large, 80-kb to 300-kb plasmids that can be exchanged between bacterial species. In many cases, these plasmids also encode other antimicrobial resistance genes. Therefore, it is common for organisms expressing an ESBL to express co-resistances to aminoglycosides, trimethoprim-sulfamethoxazole, and tetracyclines (Jacoby and Medeiros, 1991). *Salmonella* isolates that demonstrate reduced susceptibility to one or more of ceftazidime, cefuroxime, cefotaxime, ceftriaxone, cefpodoxime or aztreonam but remain susceptible to cefoxitin or cefotetan are considered as potential producers of ESBLs (Sturenburg and Mack, 2003). Expanded-spectrum cephalosporins (ESCs) such as ceftriaxone, together with fluorinated quinolones, are the choice of antibiotics in the treatment of invasive *Salmonella* infections. Resistance to ESCs among non-typhoid *Salmonella* has been recognized since the late 1980s. Currently, ESC-resistant *Salmonella* strains are reported world-wide and in some areas their incidence is significant. Resistance is mainly due to acquisition of multi-resistant plasmids encoding a variety of extended-spectrum and AmpC-type -lactamases (Miriagou *et al*., 2004).

5. Conclusions

Antibiotic resistance is an important issue affecting public health, and rapid detection in clinical laboratories is essential for the prompt recognition of antimicrobial-resistant organisms. Infection-control practitioners and clinicians need the clinical laboratory to rapidly identify and characterize different types of resistant bacteria efficiently to minimize the spread of these bacteria and help to select more appropriate antibiotics. This is particularly true for ESBL-producing bacteria. The epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community. E coli that produce CTX-M β lactamases seem to be true community ESBL producers with different behaviors from *Salmonella spp*, which produce TEM-derived and SHV-derived ESBLs. These bacteria have become widely prevalent in the community setting in certain areas of the world and they are most likely being imported into the hospital setting. A recent trend is the emergence of community-onset bloodstream infections caused by ESBL-producing bacteria, especially CTX-M-producing *Salmonella spp*. These infections are currently rare, but it is possible that, in the near future, clinicians will be regularly confronted with hospital types of bacteria causing infections in patients from the community, a scenario very similar to that of community-acquired *Salmonella spp*, The carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by ESBL-producing Enterobacteriaceae. The spread of E coli that produce CTX-M β lactamases will have important future implications for the empirical treatment of community-associated bloodstream infections, particularly in patients with associated urinary-tract infections, and therefore merits close monitoring with enhanced surveillance studies. Molecular methods for the detection of CTX-M β lactamases show potential to screen large numbers of these bacteria in a rapid fashion. We recommend that internationally funded efforts should be undertaken to track and monitor the worldwide spread of *Salmonella spp*, that produce CTX-M β lactamases within the hospital and community settings. If this emerging public-health threat is ignored, the medical community may be forced to use the carbapenems as the first choice for the empirical treatment of serious infections associated with urinary-tract infections that originate in the community. Research is warranted to determine whether significant clinical differences exist among the carbapenems, and to define the best therapy of less severe infections caused by ESBL-producing Enterobacteriaceae. We also recommend that future investigations be undertaken to study the microbiological and ecological factors that make CTX-M-producing *Salmonella spp*, such successful pathogens. This will help to prevent future infections caused by these medically important pathogens.

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