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Improved Vaccine Design and Delivery as Part of an Integrated Approach to Meet the Public Health Challenge of Typhoid Fever in Developing Countries

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Abstract

Salmonella enterica serovar Typhi (*S. typhi*) is a significant cause of typhoid fever in humans. Gastrointestinal infections with this and the closely related bacterium *S. paratyphi* that causes paratyphoid fever pose a public health challenge, particularly in developing countries where people of all age groups are commonly affected. However, limited data exist to estimate the total clinical burden of such enteric fever in Asian and African countries. Despite the demonstrated success of a number of typhoid vaccines, these are still not deployed widely enough to protect whole communities. In typhoid fever-endemic areas especially there is a pressing need to consider the introduction into routine public health programs of new generation typhoid vaccines. To this end, several novel conjugate vaccines have undergone clinical trials for human use. The optimum age at which children are immunized needs to be re-evaluated as there is a requirement for an efficacious and potent vaccine that can be used in children below the current vaccination window of two to five years of age. This review highlights the key virulence factors of *S. typhi* that are associated with antibiotic resistance and considers the need to introduce widespread public vaccination programs to help combat typhoid fever worldwide. Improvement in sanitation and water systems is the principal long-term solution to disease prevention. In addition, early diagnosis and appropriate treatment can reduce rates of morbidity and lessen disease severity in individual patients, even in regions with restricted health care facilities. In order to support national vaccination programs, in locations where typhoid fever is endemic a range of policies on public health education, good hygiene practices, increased quality of the supply of drinking water and regular monitoring of *S. typhi* antibiotic resistance patterns should be instigated.

Keywords

Salmonella; Typhoid; Fever; Disease; Virulence; Serovar; Typhi; Diagnosis; Vaccine; Vaccination; Prevention; Control

Introduction

Salmonella is named after the American bacteriologist Dr Daniel Elmer Salmon, who first isolated the Gram-negative, non-spore-forming, facultative anaerobic bacillus from porcine intestine in 1884 [1]. The genus *Salmonella* lies within the kingdom Eubacteria, class Gammaproteobacteria, order Enterobacteriales and family Enterobacteriaceae, and contains two species, *S. enterica* and *S. bongori*, each of which contains multiples serotypes (or so-called 'serovars')[2,3].

Since 1934 the antigenic formulae of *Salmonella* serovars have been maintained by the Pasteur Institute, Paris, France, at what is now the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella*. In the most recent full listing of the 'White-Kauffmann-Le Minor' scheme there are acknowledged to be 2,579 so-called 'serovars' of *Salmonella* [4]. Newly recognized serovars are also reported periodically in the journal *Research in Microbiology* [3]. The majority (59%) belong to *S. enterica* subsp. I (*S. enterica* subsp. *enterica*)[5]. Serovars within *S. enterica* subspecies II (*S. enterica* subsp. *salamae*), IIIa (*S. enterica* subsp. *arizonae*), IIIb (*S. enterica* subsp. *diarizonae*), IV (*S. enterica* subsp. *houtenae*), IV (*S. enterica* subsp. *indica*), and *S. bongori* are usually isolated from cold-blooded animals and from the environment but rarely from humans [6].

Among all subspecies *S. enterica* serovar Typhi (*S. typhi*) is a highly virulent, host-restricted, invasive facultative intracellular pathogen that infects only humans. Typhoid fever, a food- and water-borne disease caused by *S. typhi*, is a serious global health problem in developing countries. Worldwide, typhoid fever affects approximately 21.5 million people every year, of which 1-3% of cases, around 200,000-600,000, are fatal [7,8]. Due to its changing modes of presentation and development of multidrug-resistant species, typhoid fever is becoming increasingly difficult to diagnose and to treat [9].

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Paratyphoid fever, caused by *Salmonella paratyphi* serovars A, B or C, has a disease presentation similar to that of typhoid fever but its occurrence is reportedly about one quarter that of typhoid fever, approximately 5.4 million [7]. By comparison, the incidence of Non Typhoidal *Salmonella* (NTS) diseases is over 90 million cases per annum [10-12]. *S. paratyphi* A is thought to cause milder disease than does *S. typhi*, with predominantly gastrointestinal symptoms [13]. The actual global burden of 'enteric fever', the collective term used to identify disease characterized by fever and abdominal pain caused by dissemination of *S. typhi* or *S. paratyphi*, is difficult to determine because many cases are unrecognized, particularly in young children who may have a non-specific illness, while it is not a notifiable disease in endemic countries [14,15].

Disease Transmission

Transmission of the disease usually occurs via the faecal-oral route, upon ingestion of contaminated water or food, or through inadequate sanitation. Consuming raw milk products, flavoured drinks, vegetables and ice-creams from street vendors is a common source of infection [16,17]. A Pakistani study conducted in 2012 estimated the annual incidence rate of *S. typhi* to range from 252 to 503 per 100,000 child years in three impoverished areas of Karachi [18]. In addition, a retrospective review of people presenting to a US hospital emergency department with confirmed cases of *S. typhi* infection indicated that two-thirds of patients had a history of recent travel to a typhoid-endemic country prior to illness [19].

Virulence of *Salmonella* Species

Salmonella typhi has a combination of factors that makes it an effective pathogen. Virulence factors including type III secretion systems, Vi antigen, lipopolysaccharide, flagella, fimbriae, porins and heat-shock proteins (e.g. GroEL), as well as various other factors essential for the intracellular survival of *S. enterica*, have been characterized [20]. *Salmonella* has many virulence-associated genes found within clusters in its genome, which are known as *Salmonella* Pathogenicity Islands (*S*PIs). The location of these genetic islands is either on the bacterial chromosome or on plasmids, each flanked by repeat sequences, and which tend to have a varied G/C composition compared to that surrounding region [21,22]. To date, 23 *S*PIs have been described but the function of all the genes contained within each island remains to be elucidated [23,24]. The genomes of *S. typhimurium* and *S. typhi* share 11 common *S*PIs; four are specific to *S. typhi* (*S*PI-7, 15, 17 and 18) and only one (*S*PI-14) for *S. typhimurium* [25]. The *viaB* gene which is located on *S*PI-7 encodes an osmolarity regulatory protein, TviA. In addition, an adjacent operon formed by the *tviBCDE* gene encodes for biosynthesis and *vexABCDE* genes encode for surface assembly that is present solely in *S. typhi*. Moreover, it is a positive regulator of these operons that encodes functions for the biosynthesis of Vi capsular polysaccharide [26-28].

Salmonella enterica spp. contains two major pathogenesis determinants (*S*PI-1 and *S*PI-2) that encode two type III secretion systems (T3SS). T3SS contains a secretion apparatus that acts like a molecular syringe which helps the bacteria to invade epithelial cells as well as to survive inside macrophages [22,26,29,30]. It is reported that TviA protein represses genes that encode important virulence factors of *S. typhi* including flagella and type III secretion system 1 (T3SS-1), the expression of which is reduced by TviA-mediated repression of the master flagellar motility regulator FlhDC [31]. In addition, this repression enables *S. typhi* to cease flagellin expression when transferring into tissues from the intestinal lumen, thereby causing reduction in the host intestinal inflammatory response by regulating T3SS-1 expression and thus facilitating evasion of the innate immune system [26,32,33].

Vi capsular antigen of *S. typhi* can reduce complement deposition due to the fact that it does not contain free hydroxyl groups that could bind complement components, thus providing protection against non-specific antibody killing [34,35]. Furthermore, it interferes directly with host interleukin (IL)-8 inflammatory signaling cascades [36]. In addition, inactivation of *fepE* that encodes the regulator of very-long O-antigen chains results in improved function of the Vi capsular polysaccharide and increases the anti-inflammatory properties of capsular antigen [28]. However, it is reported that Vi-negative *S. typhi* isolates use alternative mechanisms to evade the immune system and are capable of adapting to new environmental conditions [37].

The virulence genes of *Salmonella* spp. also encode five different Sips (*Salmonella* invasion proteins), namely Sip A, B, C, D and E, which are capable of inducing apoptosis in macrophages [21]. In addition, *Salmonella* outer membrane vesicles (OMV) have been identified as another means used to transfer its virulence factors into the cytoplasm of a host cell [38]. Moreover, plasmids that are associated with virulence have been identified in *S. typhi*, such as the chimeric plasmid pRST98 that carries genes which are involved in drug resistance and apoptosis induction in macrophages [39]. The role of plasmids carrying antimicrobial resistance genes, such as *cat*, *dhfr7*, *dhfr14*, *sul1* and bla_{TEM-1}, in the transfer and spread of antimicrobial resistance has been well described [24].

Typhoid Toxin

Three types of bacterial genotoxins have been identified: (i) protein toxins, e.g. cytolethal distending toxin (CDT), produced by several Gram-negative bacteria, such as *Aggregatibacter actinomyc -etemcomitans*, *Haemophilus ducreyi*, *Providencia alcalifaciens*, Campylobacter spp., *Helicobacter* spp., *Shigella* spp. and *Yersinia* spp. [40,41]; (ii) typhoid toxin, produced by the facultative intracellular pathogen *S. typhi*; and (iii) colibactin, produced by strains belonging to the phylogenetic group B2 of *Escherichia coli* [42].

The typhoid toxin is encoded by both typhoidal species, *S. typhi* and *S. paratyphi* [43-45]. However, it was thought initially not to be produced by NTS but has since been identified in at least 40 serovars [46]. It is a unique member of the AB_5 exotoxin family, each of which comprises a catalytic A-subunit, responsible for disruption of essential host functions, and a pentameric B-subunit that binds to specific glycan receptors on the target cell surface [47].

Mode of action

Typhoid toxin is an A_2B_5 toxin, composed of two covalently-linked enzymatic subunits, the deoxyribonuclease (encoded by *cdtB*, cytolethal distending toxin subunit B) and the ADP ribosyltransferase (encoded by *pltA*, pertussis-like toxin subunit A), associated with the single homopentameric B subunit (encoded by *pltB*, pertussis-like toxin subunit B). In fact, typhoid toxin appears to have evolved from a combination of two exotoxins, cytolethal distending and pertussis toxins. The mode of action of PItA has not yet been identified whereas the CdtB subunit is known to cause DNA damage and cell cycle arrest. In addition, CdtB protein shows sequence homology to human cells and bacterial enzymes such as DNase I [48,49]. In *S. typhi*, the CdtB islet includes five genes, namely *pltA*, *pltB*, *ttsA*, *sty1887* and *cdtB*. However, *pltA* and *pltB* encode homologues of pertussis toxin components, which are responsible for ADP-ribosylation of a host protein and export of the CdtB subunit from the bacterium-containing vacuole as well as from infected host cells [50].

The main sites of typhoid toxin activity are sialylated glycan moieties on the host cell receptor through which the B subunit of PltB selectively recognizes and binds. Furthermore, sialoglycans on human cells are different from those on cells of other animals because they display N-acetylneuraminic acid (Neu5Ac). Thus, typhoid toxin is cytotoxic and targets specifically those cells exhibiting Neu5Ac glycans on their surface [49,51,52]. A recent study suggested that typhoid toxin binds to a variety of cell receptors, including PODXL and CD45 on B and T cells and is able to intoxicate a number of different cell types. In experimental animals, the typhoid toxin can reproduce many of the pathognomonic symptoms of typhoid fever [49].

The typhoid toxin produced by *Salmonella* spp. differs from that of the CDT produced by other Gram-negative bacteria. The key differences include: (i) production of typhoid toxin occurs only within host eukaryotic cells; (ii) transport of this toxin to the extracellular environment occurs by a unique mechanism that involves vesicle carrier intermediates, after which the exported toxin may either re-enter the cell or intoxicate a nearby cell; (iii) typhoid toxin A_2B_5

structure requires a reducing atmosphere to dissociate the two subunits, PltA and CdtB; (iv) the receptors to bind to the host cell utilize PItB rather than CdtA and CdtC subunits [49,53].

Typhoid toxin is an important virulent factor of *S. typhi* that plays a crucial role in the pathogenesis of typhoid fever. The observations described warrant further detailed study in animal models that may facilitate vaccine development and a greater understanding of typhoid fever pathophysiology.

Pathogenesis of *Salmonella*

Salmonella typhi is a motile, non-lactose fermenting bacillus that can produce hydrogen sulphide gas [54]. This bacterium invades the gastrointestinal mucosa and translocates to the lymphoid follicles, where it survives and multiplies intracellularly within mononuclear phagocytes, and then disseminates via the bloodstream to the liver, spleen, bone marrow, gall bladder and/or intestinal lymph nodes. Hence, *S. typhi* is recognised to be a facultative intracellular pathogen [55-57].

The typical incubation period for typhoid serovers is 8-14 days, while the average incubation period is 14 days and symptoms of clinical infection can persist for up to three weeks [58]. The classical symptoms includes gradual onset of sustained fever, chills, hepatosplenomegaly and abdominal pain. Although fever spike (39-40 °C) is ultimately an important feature of typhoid, progression of the disease is relatively slow and it is very rare for death to occur due to sepsis or cytokine excess [59]. Patients may typically experience rash, nausea, anorexia, diarrheoa or constipation, headache, relative bradycardia and reduced level of consciousness. Potential complications include intestinal perforation or bleeding, encephalopathy and focal metastatic complexities such as cholecystitis or hepatitis [60]. Serious complications include septicaemia and meningitis, most cases of which are observed in paediatric and immunocompromised patients [61]. Without effective treatment (as was the case prior to the advent of antibiotics) typhoid fever has a fatality rate of 10-30%, a number that declines to 1-4% with provision of appropriate therapy [62].

Bacterial internalization is determined by a complex interplay of both host and pathogen factors. *S. typhi* can survive inside a variety of cells including macrophages, dendritic cells, neutrophils, microfold (M) cells and enterocytes. This bacterium is also able to induce a process of phagocytosis in non-phagocytic cells such as epithelial cells via the action of SPI1-T3SS [63]. Changes occur in the plasma membrane of the host cell that enable ingestion into a membrane-bound vesicle by phagocytosis or *Salmonella*-mediated invasion. Rho family GTPases and phosphoinositides are involved in the cell signal transduction pathway, actin remodeling and membrane trafficking [30,64]. This invasion process is also facilitatedby the help of flagella-based motility [65].

After invading epithelial cells from the apical side, each bacterium resides and replicates within a membrane-bound vacuole, known as the *Salmonella*-Containing Vacuole (SCV). Following penetration of M cells, bacteria access the underlying structure of the lymphoid tissue, which is an area rich in phagocytic cells and that serves as the initial site of intracellular infection [66]. Both cellular and antibody-mediated immunity are known to play roles in controlling and clearing typhoid infection. In fact, the clinical symptoms of typhoid fever may be initiated by a strong cell-mediated immune response that releases typhoid bacilli or fragments thereof from macrophages of the reticuloendothelial system [67].

Chronic Carriers

Being a chronic carrier is a contagious state that can occur following symptomatic or subclinical infections of *S. typhi*. Fortunately, after appropriate treatment, the majority of patients recover from the acute phase of typhoid fever; however, 3-5% of individuals who are infected with *S. typhi* develop a chronic infection of the gall bladder [68,69]. Ultimately, these chronic carriers provide a crucial reservoir for further spread of the disease through bacterial shedding in faeces and urine [13,70,71]. Chronic infections can persist for decades. It is quite difficult to identify carriers because 25% of such persons do not show any clinical manifestations during the acute phase of disease [72]. Long-term carriage of *S. paratyphi* is less characterized than that of *S. typhi* but recent findings suggest an equal prevalence of serovars Typhi and Paratyphi A in Nepal and other endemic locations [73].

Mechanism of Antibiotic Resistance

There are various mechanisms through which *S. typhi* can develop resistance to antibiotics including chromosomal DNA-mediated resistance, the presence of plasmid HI1, decreased permeability and efflux pumps [74]. Genomic islands are relatively larger DNA segments that also contribute to antibiotic resistance as well as carrying genes for additional functions such as metabolic activities, pathogenicity and symbiosis. These genomic islands can be acquired via horizontal gene transfer [75]. Similarly, SGI11 is a *Salmonella* genomic island, identified for the first time in multidrug-resistant (MDR) *S. typhi*. For example, SGI11-carrying *S. typhi* possessing seven resistance genes ($bla_{\text{TEM-1}}$, *catA1*, *strA*, *strB*, *sul1*, *sul2* and *dfrA7*) conferred resistance to five first-line antimicrobials [76]. Thus, further research is needed to monitor chromosome-mediated resistance to first-line antibiotics, especially fluoroquinolones, for the treatment of typhoid fever. Moreover, different mechanisms have been found among *Salmonella* species to determine quinolone resistance, which is often associated with mutations in the quinolone resistance-determining region (QRDR) including genes DNA gyrase (*gyrA* and *gyrB*) or topoisomerase (*parC* and *parE*) [77]. For example, mutations in the *gyrA* and secondary mutations in *parC* resulted in elevated minimum inhibitory concentrations and reduced susceptibility to fluoroquinolones in *S. typhi* [78-80].

Plasmids that mediate quinolone resistance (PMQR), including *qnr* (*qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD*), *qepA*, *oqxAB* and *aac(6')-Ib-cr*, are present in *Salmonella* species that show resistance to quinolones but only *qnrB*, *qnrS* and *aac(6')-Ib-cr* (aminoglycoside acetyltransferase) genes have been described in *S. typhi* [81-85]. Efflux pumps and decreased permeability have also been associated with quinolone resistance by *Salmonella* [86-88].

Current State of Antibiotic Resistance

The emergence and increased number of MDR *S. typhi* strains, their persistence and transmission in many Asian countries, are due principally to a combination of urbanization, migration, travelling and trade [79,80,89,90]. Almost 80% of patients infected with MDR *S. typhi* strains originate from Asia and the remainder occurs mostly in Africa and Latin America [91,92].

A retrospective examination of the epidemiology of a massive *S. typhi* outbreak in Zambia during 2010-12 revealed a high level of resistance to first-line antimicrobials used for the treatment of typhoid fever [93]. 83% of isolates were resistant to five antimicrobial drug classes, aminoglycosides, β-lactams, phenicols, sulfonamides and trimethoprim, and which were thereby classified as being MDR. *S. typhi* haplogroup H58, containing IncHI1 plasmid, has emerged globally and is responsible for the majority of typhoid infections in Southeast Asia and sub-Saharan Africa, indicative of intercontinental spread of MDR isolates [94-97]. All H58 MDR *S. typhi* isolates investigated in Kenyan typhoid cases contained large self-transmissible IncHI1 ST6 plasmids, which has been the most prevalent haplotype (87%) in Kenya since the early 2000s [98,99]. Likewise, from 1995 onwards 98% of MDR *S. typhi* isolates were of the same H58 haplotype, carrying PST6 plasmid, which conferred an advantage of growth in high-salt content medium in Bangladesh [100]. In contrast, in the West African nation of Guinea no MDR H58 haplotypes were recovered but rather MDR quinolone-susceptible isolates carried a 190-kb incHI1 pST2 plasmid or a 50-kb IncN pST3 plasmid [101].

Interestingly, it was found that resistance genes of the H58 strain are integrated into the bacterial chromosome and are not carried on a plasmid [98,102]. The vast majority of *S. typhi* incidence, almost 98% of cases, isolated from the Mekong river delta of Vietnam was caused exclusively by a H58 nalidixic acid-resistant haplotype conferred by an identical mutation in *gyrA* [98]. The rapid emergence since

2000 of the H58 lineage of *S. typhi* in Blantyre, Malawi, is likely to be associated with the widespread and improper use of antimicrobials that maintains this phenotype [103].

Recent studies have shown that high level fluoroquinolone-resistant *S. typhi* H58 has emerged in Nepal and Bangladesh, suggesting that fluoroquinolones are no longer effective to treat this haplotype [76,104,105]. Intensive surveillance is needed to monitor the spread of chromosome-mediated MDR and fluoroquinolone-resistant *S. typhi* strains in these regions. In contrast, the dominance and resistance to fluoroquinolones of H58 haplotype is thought not to be driven solely by continued use of this drug and that the frequency of resistance may escalate further even if the use of this drug is restricted. In fact, the S83F mutation is responsible for the evolution of fluoroquinolone-resistant *S. typhi* [106]. The use of a disk susceptibility test for screening of nalidixic acid or ciprofloxacin and ofloxacin resistance has been proposed as a means to detect *S. typhi* isolates with reduced fluoroquinolone susceptibility that will assist clinicians in the choice of therapy [80]. This potentially poses an increasing threat to treatment with existing antibiotics and there is an urgent need to understand the spread of this haplotype and how it may be overcome.

The increased rate of nalidixic acid-resistant *Salmonella* spp. associated with reduced susceptibility to fluoroquinolones has been demonstrated recently in Egypt, Uzbekistan, Pakistan, Qatar, Jordan and Iraq [90,107]. There is growing evidence to show that MDR *S. typhi* strains are increasing worldwide [74,99]. A study conducted in Canada between 2002-07 revealed 18% MDR *S. typhi* isolates and showed higher levels of resistance (80%) to nalidixic acid, whereas no ciprofloxacin resistance was observed [108].

Likewise, the re-emergence of the conventional first-line antibiotics ampicillin, chloramphenicol and cotrimoxazole for treatment of typhoid fever due to raised susceptibility of *S. typhi* and *S. paratyphi* A to these drugs has been reported [109-111]. An observational study was conducted in Lebanon during 2006-08 based on blood culture positivity for *S. typhi*. This confirmed that antimicrobial resistance among *S. typhi* isolates remained rare and that first-line drugs should still be considered as an appropriate therapeutic choice in patients with typhoid fever [112].

The most reliable and effective drug class for treating typhoid fever is the third generation cephalosporins, the use of which was advocated by the WHO as long ago as 2003 [57,113]. Recent findings showed that almost all *Salmonella* isolates were susceptible (99.7%) to ceftriaxone (a third-generation cephalosporin) [114]. This remains the realistic choice as an alternative treatment for typhoid fever and is also recommended against MDR and nalidixic acid-resistant *S. typhi* isolates [90,115].

Extended spectrum β-lactamase (ESBL)-producing *S. typhi* has only been reported from Bangladesh, Egypt, India, Iran, Iraq, Pakistan and the Philippines [116]. Various other studies from India, Iraq and the Philippines confirmed the presence of this strain and also genes for ESBL producing *S. typhi* have been sequenced [93]. The spread of resistance calls for globally restricted use while an international antimicrobial surveillance system will be crucial for determining the reliability of antibiotic susceptibility testing in endemic areas.

It is reported that *S. typhi* contains genes bla_{CTX-15} and *qnrB2,* shows resistance to cephalosporins as well as reduced quinolone susceptibility that can transfer easily by conjugation into *Escherichia coli* [82]. Similarly, the dissemination of the New Delhi metallo-β-lactamase (NDM)-1 enzyme in enteric organisms is alarming due to the risk of NDM-1 transmission to *Salmonella* species that will limit further the therapeutic options for treatment [117].

Salmonella Vaccines

Historically, considerable effort has been invested by many scientists to develop an effective vaccine against *Salmonella* but currently only two licensed vaccines are commercially available to combat typhoid fever – a purified Vi polysaccharide parenteral vaccine and the live oral attenuated Vi negative *galE* mutant *S. typhi* strain Ty21a vaccine (marketed as Vivotif by Berna Biotech)[118]. The WHO recommends the use of typhoid vaccines for communities living in regions where the disease is endemic and for high-risk populations such as travelers, laboratory workers and food handlers with carrier status [119,120]. In the past two decades, public health officials have debated the best methods of evaluating typhoid vaccine effectiveness and whether the focus on vaccination coverage is a distraction from underlying issues of improvements in water supply, sanitation and hygiene[121].

Inactivated whole-cell vaccine: In 1896, heat-treated, phenol-preserved and acetone-killed lyophilized injectable whole-cell *S. typhi* vaccine was generated and administered in England and Germany. The efficacy of this vaccine was assessed and thereafter remained in use in several countries [122,123]. It was first licensed in 1952 by Wyeth but was associated with high rates of fever and systemic reactions and production was discontinued in 2000 [124]. After the introduction of licensed typhoid vaccines, it was gradually replaced by the parenteral killed whole-cell vaccine as the recommended prevention for typhoid fever. These two vaccines mediate protection by different mechanisms. Parenteral Vi vaccine elicits anti-Vi antibody in serum and it is T-cell independent, so does not stimulate T helper (Th) cells that could enhance and broaden the immune response and elicit immunological memory [125]. In contrast, Ty21a does not express Vi and therefore does not elicit anti-Vi antibody and is likely to mediate protection by serum and mucosal antibodies raised to other antigens of *S. typhi* [118,126] and by cell-mediated immune responses, including cytotoxic T cells at systemic and mucosal levels [127-131]. Although Ty21a is known to confer a moderate level of long-lived protection (typically 5-7 years) it requires three or four doses for optimal immunogenicity [132].

Live, attenuated Ty21a oral vaccine: Despite considerable research into typhoid vaccination, there is little information on the protective immunological mechanisms elicited by oral immunization with *S. typhi*. Ty21a vaccine has been shown to have a variable rate of protection depending on the formulation used and the number and spacing of the doses administered. The current recommended vaccination protocol with the live, attenuated Ty21a vaccine consists of one enteric-coated capsule taken on alternate days (day 0, 2, 4 and 6) for a total of four capsules. The Swiss manufacturer of Ty21a recommends revaccination with the entire four-dose series every five years if continued or renewed exposure to *S. typhi* is expected [133]. It was found out that under conditions of moderate transmission of typhoid fever, a liquid formulation of the Ty21a oral vaccine had a protective efficacy of 96% in Egypt, while an enteric coated capsule formulation had an efficacy of 67% in Chile. However, in Indonesia, when these two formulations were compared, the protective efficacies of liquid and enteric coated vaccines were 53% and 42%, respectively [134,135]. Furthermore, the liquid formulation provided protection in young children (5-9 years), with 82.3% efficacy, and in older children, with 69.3% efficacy, whereas the capsules significantly protected only the older cohort [136]. At present, neither vaccine is registered for administration to infants less than two years of age [134,137].

In general, people living in endemic regions are more susceptible to infection. Several other factors also contribute to a vaccine's efficacy, such as the host's socioeconomic status, genetic make-up, nutritional state, previous exposure to related infectious agents, and composition of their intestinal microbiota [138]. Moreover, immunization with either a single dose or a four-dose vaccination of Ty21a does not disrupt the composition, diversity or stability of the bacterial community in the intestinal flora [139].

There are several advantages of oral vaccines including excellent clinical acceptability, ease of administration, ease of mass immunization, and long-term efficacy sustained over at least seven years [132]. There is no vaccine available for prevention of non-typhoidal *Salmonella*, although many studies have reported that Ty21a and Vi vaccines both elicit a cross-reactive response to *S. paratyphi* A and B [140]. This suggests that concomitant administration of these two vaccines may potentially enhance cross-protection against *S. paratyphi* A and B, but further confirmatory studies are required [140].

The human immune response to *S. typhi* is very complicated and involves diverse components of the immune system such as localized, systemic antibody and cell-mediated immunity. Antibodies to *S. typhi* antigens in the serum (e.g. Vi and lipopolysaccharide O) play an important role in defence against typhoidal bacteraemia caused by extracellular bacteria. However, because *S. typhi* can enter and multiply inside mononuclear phagocytes of Peyer's patches, then spread to phagocytes within the liver, gall bladder and spleen, thereby avoiding destruction by antibodies and complement, cell-mediated immunity is considered essential to eliminating *S. typhi* infection [141]. Moreover, the increased resistance that develops after primary infection or vaccination needs T cell-mediated cytokines such as interferon (IFN)-γ, tumour necrosis factor (TNF)-α and IL-12 in addition to opsonizing antibody [142]. In 2002, Lundin et al. demonstrated an oral vaccine-stimulated large increase in both the proliferation and the production of IFN-γ by peripheral blood T cells [129]. The major response was observed among both $CD4^+$ and $CD8^+$ T cells, but most IFN-γ was produced by $CD8^+$ cells within 7-14 days of the onset of vaccination [128].

It was observed previously that immunization with attenuated *S. typhi* strains, including Ty21a, CVD 906, CVD 908, CVD 908-*htrA* and CVD 909, results in the appearance in peripheral blood of sensitized lymphocytes that exhibit significantly increased lymphoproliferative responses and Th1-type cytokine production patterns in response to *S. typhi* antigens [143-149]. In addition, these strains have been used as vectors to express a variety of heterologous antigens. Ultimately, improved oral typhoid vaccines have been developed that can trigger protective immunity in humans to both *S. typhi* itself and to carried foreign antigens for which it acts as a vaccine carrier.

Vi polysaccharide vaccine: The Vi antigen is thought to be a major virulence factor and protective antigen of *S. typhi* [150]. Vaccines based on the Vi polysaccharide of *S. typhi* are safe, immunogenic and are licensed for human use. In 1986, Vi polysaccharide typhoid vaccine (Vi vaccine) was evaluated for its ability to prevent typhoid fever in a pilot study in Nepal. No significant side-effects of Vi vaccination were observed and it conferred between 55-75% protection against typhoid fever in endemic regions [125,151]. This parenterally administered vaccine is approved for use in adults and children over two years of age [152]. Similarly, Vi capsular polysaccharide vaccine showed a significant level of total protection in children 5-16 years of age, which is consistent with other studies of capsular vaccines conducted in India, Nepal, China and South Africa [18].

There are several advantages of Vi vaccine administration. First, it is given in a single dose intramuscularly, which is well tolerated. Second, Vi is putatively more thermostable than Ty21a, which helps to reduce the economic burden on developing countries of providing cold temperature storage facilities. Third, it may be possible to successfully transfer the production technology for the Vi vaccine to pharmaceutical companies in typhoid fever-endemic countries [134,153].

In China, Vi vaccine conferred 71% protection at 12-month follow-up in randomized controlled trials [154]. A retrospective analysis conducted to investigate long-term protection following immunization indicated that Vi vaccine protects for at least two years, and in some vaccines this was sustained for three years [155]. In locations in the Indian subcontinent where typhoid fever is endemic, such as Kolkata and Karachi, the Vi capsular polysaccharide vaccine is immunogenic in children aged 2-16 years, conferring protection for two years as detected by a rise in the titre of antibodies. However, immune responses are greater in older children (5-16 years) than in younger children (2 to < 5 years) [156]. Thus, a single dose of Vi can provide moderate protection for a limited duration, but this vaccine has certain limitations including poor immunogenicity in infants and young children, short-lived immunity and a lack of anamnestic immune responses to subsequent doses [157]. Therefore, re-vaccination every three years is recommended for sustained protection against typhoid fever in high-risk populations [158].

This vaccine contains purified Vi polysaccharides of *S. typhi* that trigger a principally humoral response, protection being mediated by Vi-specific IgG antibodies [156,159]. These are elicited in 85-95% of vaccines after Vi capsular polysaccharide vaccine administration [160]. One study reported enhanced O-antigen-specific responses in Vi vaccinated volunteers, assuming this was caused by trace amounts of lipopolysaccharide remaining after the purification step of vaccine preparation. It is feasible that part of the immunity conferred by the Vi vaccine may, in fact, be contributed by a response to typhoidal O-antigens [161].

Vi polysaccharide-protein conjugate vaccines: In order to overcome the limitations of age-related and T cell-independent immunogenicity of the vaccine, it is important to conjugate the Vi polysaccharide with a protein to acquire the desired immunogenicity. Conjugation of capsular polysaccharides with carrier proteins renders them immunogenic in infants and capable of eliciting memory, booster responses and inducing IgG antibodies [150].

An alternative Vi-conjugate vaccine, Vi-CRM_{197}, based on conjugation of Vi polysaccharide from *Citrobacter* to a mutated, non-toxic diphtheria toxin carrier protein, has also shown excellent immunogenicity when tested in adults [162]. Further studies are in progress to evaluate this vaccine in small children in Southeast Asia [152].

A further conjugate vaccine under development, named *S. typhi* Vi O-acetyl pectin-rEPA conjugate vaccine, is a modified conjugate vaccine in which Vi-PS is conjugated to a non-toxic recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA). This has been shown to be safe and immunogenic in children 2-4 years of age with efficacy of 90% [163,164], but to date has been evaluated in only one clinical trial [137].

Another prototype recombinant attenuated *S. typhi* vaccine that is undergoing clinical trials, RASTyV, expresses a heterologous antigen, *Streptococcus pneumoniae* surface protein PspA and rpoS, to prevent pneumococcal diseases in infants and children [165]. In 2013, a conjugated vaccine named Vi-tetanus toxoid was licensed in India for delivery to all age groups and this new generation of typhoid vaccine opens up a new era for typhoid fever prevention and elimination [166].

Conclusion

Although examination of blood cultures remains a gold standard for diagnosis of typhoid fever, it is poorly sensitive, necessitates multiple samples and results are often delayed. Culture of bone marrow aspirate is acknowledged to be more sensitive but this requires equipment, consumable supplies and experienced laboratory staff rarely found in primary healthcare settings in the developing world. Rapid antibody tests are constantly in use in many microbiology laboratories of low economic countries to facilitate quick diagnosis and efficient disease management of typhoid fever. It is suggested that novel diagnostic tests should be developed that offer greater specificity, sensitivity and cost effectiveness. In endemic regions, improved and affordable diagnosis should complement policies on public health education, good hygiene practices, long-term improvement in the supply of drinking water, regular monitoring of bacterial resistance patterns and, in particular, preventive vaccinations.

In spite of the demonstrated success of a number of established typhoid vaccines, their collective deployment is not sufficient to protect large and widespread populations. In regions where typhoid fever is endemic, there is an urgent need to consider the introduction of new generation vaccines into public health programs and thus several conjugated vaccines have undergone clinical trials for human use. In different studies the age of greatest susceptibility to typhoid fever of children varies from 10-15 years to aged > 1-5 years, while there is a marked variation in the count observed in rural and urban areas. Thus, the optimum age for immunization requires re-evaluation in order to achieve an efficacious vaccine that provides strong humoral and cellular immunity against typhoid fever in children of all ages.

Conflict of Interest

The authors declare that they have no competing issues of interest.

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References

1. Smith T. The hog-cholera group of bacteria. US Bur Anim Ind Bull. 1894;6:6-40.
2. Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. *Salmonella* nomenclature. J Clin Microbiol. 2000;38:2465-2467.
3. Issenhuth-Jeanjean S, Roggentin P, Mikoleit M, Guibourdenche M, de Pinna E, et al. Supplement 2008-2010 (no. 48) to the White-Kauffmann-Le Minor scheme. Res Microbiol. 2014 Sep;165(7):526-530.
4. Grimont AD, Weill F-X. Antigenic formulae of the *Salmonella* serovars, 9th ed. World Health Organization Collaborating Centre for Reference and Research on *Salmonella* (Pasteur Institute, Paris, France); 2007.
5. Su LH, Chiu CH. *Salmonella*: clinical importance and evolution of nomenclature. Chang Gung Med J. 2007 May;30:210-218.
6. Farmer JJ, McWhorter AC, Brenner DJ, Morris GK. The *Salmonella*-Arizona group of Enterobacteriaceae: nomenclature, classification, and reporting. Clin Microbiol Newslett. 1984 May;6:63-66.
7. Typhoid vaccines: WHO position paper. Wkly Epidemiol Rec. 2008 Feb;83(6):49-59.
8. Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale VV, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health. 2014 Oct;2:e570-e580.
9. Harish B N, Menezes G A. Antimicrobial resistance in typhoidal salmonellae. Indian J Med Microbiol. 2011;29:223-229.
10. Arya SC, Sharma KB. Urgent need for effective vaccine against *Salmonella paratyphi* A, B and C. Vaccine. 1995;13:1727-1728.
11. Sood S, Kapil A, Dash N, Das BK, Goel V, et al. Paratyphoid fever in India: an emerging problem. Emerg Infect Dis.1999;5:483-484.
12. Majowicz SE, Musto J, Scallan E, Angulo F J, Kirk M, et al. International collaboration on enteric disease 'Burden of Illness' studies, the global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis. 2010;50:882-889.
13. Bhan MK, R Bhal, S Bhatnagar. Typhoid and paratyphoid fever. Lancet. 2005;366:749-762.
14. Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, et al. Typhoid fever in children aged less than 5 years. Lancet. 1999 Aug;354:734-737.
15. Khan MI, Franco-Paredes C, Sahastrabuddhe S, Ochiai RL, et al. Res Rep Trop Med. 2017;8:37-44.
16. Comeau JL, Tran TH, Moore DL, Phi CM, Quach C. *Salmonella enterica* serotype Typhi infections in a Canadian pediatric hospital: a retrospective case series. CMAJ Open. 2013 May;1:E56-E61.
17. Luby SP, Faizan MK, Fisher-Hoch SP, Syed A, Mintz ED, et al. Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. Epidemiol Infect. 1998 Mar;120:129-138.
18. Khan MI, Ochiai RL, Soofi SB, Von-Seidlein L, Khan MJ, et al. Risk factors associated with typhoid fever in children aged 2-16 years in Karachi, Pakistan. Epidemiol. Infect. 2012;140:665-672.
19. Hoffner RJ, Slaven E, Perez J, Magana RN, Henderson SO. Emergency department presentations of typhoid fever. J Emerg Med. 2000;19:317-321.
20. Tang SW, Abubaker S, Devi S, Puthucheary S, Pang T. Induction and characterization of heat shock proteins of *Salmonella typhi* and their reactivity with sera from patients with typhoid fever. Infect Immun. 1997 Jul;65:2983-2986.
21. Kaur J, Jain SK. Role of antigens and virulence factors of *Salmonella enterica* serovar Typhi in its pathogenesis. Microbiol Res. 2012 Apr;167:199-210.
22. De Jong HK, Parry CM, Van der Poll T, Wiersinga WJ. Host-pathogen interaction in invasive Salmonellosis. PLoS Pathog. 2012 Oct;8:e1002933.
23. Hayward MR, Jansen VAA, Woodward MJ. Comparative genomics of *Salmonella enterica* serovars Derby and Mbandaka, two prevalent serovars associated with different livestock species in the UK. BMC Genomics. 2013 May;14:365.
24. Sabbagh SC, Forest CG, Lepage C, Leclerc JM, Daigle F. So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars Typhimurium and Typhi. FEMS Microbiol Lett. 2010 Apr;305(1):1-13.
25. Hurley D, McCusker MP, Fanning S, Martins M. *Salmonella*-host interactions – modulation of the host innate immune system. Front Immunol. 2014 Oct;5:481.
26. Winter SE, Winter MG, Godinez I, Yang HJ, Rüssmann H, et al. A rapid change in virulence gene expression during the transition from the intestinal lumen into tissue promotes systemic dissemination of *Salmonella*. PLoS Pathog. 2010 Aug;6:e1001060.
27. Wetter M, Goulding D, Pickard D, Kowarik M, Waechter CJ, et al. Molecular characterization of the *viaB* locus encoding the biosynthetic machinery for Vi capsule formation in *Salmonella* Typhi. PLoS One. 2012;7:e45609.
28. Crawford RW, Wangdi T,Spees AM, Xavier MN, Tsolis RM, et al. Loss of very-long O-antigen chains optimizes capsule-mediated immune evasion by *Salmonella enterica* serovar Typhi. MBio. 2013;4:e00232-13.
29. Daigle F. Typhi genes expressed during infection or involved in pathogenesis. J Infect Dev Ctries. 2008 Dec;2:431-437.
30. Malik-Kale P, Jolly CE, Lathrop S, Winfree S, Luterbach C, et al. *Salmonella* – at home in the host cell. Front Microbiol. 2011 Jun;2:125.
31. Winter SE, Winter MG, Thiennimitr P, Gerriets VA, Nuccio SP, et al. The TviA auxiliary protein renders the *Salmonella enterica* serotype Typhi RcsB regulon responsive to changes in osmolarity. Mol Microbiol. 2009 Oct;74(1):175-193.
32. Tran QT, Gomez G, Khare S, Lawhon SD, Raffatellu M, et al. The *Salmonella enterica* serotype Typhi Vi capsular antigen is expressed after the bacterium enters the ileal mucosa. Infect Immun. 2010;78: 527-535.
33. Winter SE, Winter MG, Poon V, Keestra AM, Sterzenbach T, Faber F, et al. *Salmonella enterica* serovar Typhi conceals the invasion-associated type three secretion system from the innate immune system by gene regulation. PLoS Pathog. 2014 Jul;10: e1004207.
34. Wilson RP, Winter SE, Spees AM, Winter MG, Nishimori JH, et al. The Vi capsular polysaccharide prevents complement receptor 3-mediated clearance of *Salmonella enterica* serotype Typhi. Infect Immun. 2011;79:830-837.
35. Hart PJ, O'Shaughnessy CM, Siggins MK, Bobat S, Kingsley RA, et al. Differential killing of *Salmonella enterica* serovar Typhi by antibodies targeting Vi and lipopolysaccharide O:9 antigen. PLoS One. 2016;11:e0145945.
36. Raffatellu M, Chessa D, Wilson RP, Dusold R, Rubino S, et al. The Vi capsular antigen of *Salmonella enterica* serotype Typhi reduces Toll-like receptor-dependent interleukin-8 expression in the intestinal mucosa. Infect Immun. 2005 Jun;73:3367-3374.
37. Liaquat S, Sarwar Y, Ali A, Haque A. Comparative growth analysis of capsulated (Vi+) and acapsulated (Vi-) *Salmonella typhi* isolates in human blood. EXCLI J. 2015 Feb;14:213-219.

38. Yoon H, Ansong C, Adkins JN, Heffron F. Discovery of *Salmonella* virulence factors translocated via outer membrane vesicles to murine macrophages. Infect Immun. 2011;79:2182-2192.

39. Wu S, Li Y, Xu Y, Li Q, Chu Y, et al. A *Salmonella enterica* serovar Typhi plasmid induces rapid and massive apoptosis in infected macrophages. Cell Mol Immunol. 2010;7:271-278.

40. Guerra L, Cortes-Bratti X, Guidi R, Frisan T. The biology of the cytolethal distending toxins. Toxins. 2011;3:172-190.

41. Gargi A, Tamilselvam B, Powers B, Prouty MG, Lincecum T, et al. Cellular interactions of the cytolethal distending toxins from *Escherichia coli* and *Haemophilus ducreyi*. J Biol Chem. 2013;288:7492-7505.

42. Grasso F, Frisan T. Bacterial genotoxins: merging the DNA damage response into infection biology. Biomolecules. 2015 Aug;5:1762-1782.

43. Galan JE. Typhoid toxin provides a window into typhoid fever and the biology of *Salmonella* Typhi. Proc Natl Acad Sci USA. 2016 Jun;113:6338-6344.

44. Den Bakker HC, Switt AIM, Govoni G, Cummings CA, Ranieri ML, et al. Genome sequencing reveals diversification of virulence factor content and possible host adaptation in distinct subpopulations of *Salmonella enterica*. BMC Genomics. 2011 Aug;12:425.

45. Mezal EH, Bae D, Khan AA. Detection and functionality of the CdtB, PltA, and PltB from *Salmonella enterica* serovar Javiana. Pathog Dis. 2014;72:95-103.

46. Suez J, Porwollik S, Dagan A, Marzel A, Schorr YI, et al. Virulence gene profiling and pathogenicity characterization of non-typhoidal *Salmonella* accounted for invasive disease in humans. PLoS One. 2013;8(3):e58449.

47. Beddoe T, Paton A, Le Nours J, Rossjohn J, Paton J. Structure, biological functions and applications of the AB5 toxins. Trends Biochem Sci. 2010 Mar;35:411-418.

48. De Rycke J, Oswald E. Cytolethal distending toxin (CDT): a bacterial weapon to control host cell proliferation? FEMS Microbial Lett. 2001 Sep;203:141-148.

49. Song J, Gao X, Galán JE. Structure and function of the *Salmonella* Typhi chimaeric A_2B_5 typhoid toxin. Nature. 2013;499:350-354.

50. Spano S, Ugalde JE, Galán JE. Delivery of a *Salmonella* Typhi exotoxin from a host intracellular compartment. Cell Host Microbe. 2008 Jan;3:30-38.

51. Varki N, Strobert E, Dick EJ, Benirschke K, Varki A. Biomedical differences between human and nonhuman hominids: potential roles for uniquely human aspects of sialic acid biology. Annu Rev Pathol. 2011;6:365-393.

52. Deng L, Song J, Gao X, Wang J, Yu H, et al. Host adaptation of a bacterial toxin from the human pathogen *Salmonella* Typhi. Cell. 2014 Dec;159:1290-1299.

53. Hodak H, Galán JE. A *Salmonella* Typhi homologue of bacteriophage muramidases controls typhoid toxin secretion. EMBO Rep. 2013;14:95-102.

54. Levinson W. Review of Medical Microbiology and Immunology. 13th ed. New York: McGraw-Hill Education; 2014.

55. Gordon MA. *Salmonella* infections in immunocompromised adults. J Infect. 2008;56:413-422.

56. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. Front Microbiol. 2014;5:391.

57. Butler T. Treatment of typhoid fever in the 21st century: promises and shortcomings. Clin Microbiol Infect. 2011 Jul;17:959-963.

58. Wangdi T, Winter SE, Bäumler AJ. Typhoid fever: "you can't hit what you can't see". Gut Microbes. 2012;3:88-92.

59. Charles RC, Sheikh A, Krastins B, Harris JB, Bhuiyan MS, etal. Characterization of anti-*Salmonella enterica* serotype Typhi antibody responses in bacteremic Bangladeshi patients by an immunoaffinity proteomics-based technology. Clin Vaccine Immunol. 2010;17:1188-1195.

60. Judd MC, Mintz ED. CDC Health Information for International Travel. Chapter 3, Infectious Diseases Related to Travel; Typhoid & Paratyphoid Fever; 2018.

61. Gonzalez-Escobedo G, Marshall JM, Gunn JS. Chronic and acute infection of the gall bladder by *Salmonella* Typhi: understanding the carrier state. Nat Rev Microbiol. 2011 Nov;9:9-14.

62. Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. J Glob Health. 2012 Jun;2:010401.

63. Swart AL, Hensel M. Interactions of *Salmonella enterica* with dendritic cells. Virulence. 2012 Nov;3:660-667.

64. Scott CC, Dobson W, BotelhoRJ, Coady-Osberg N, Chavrier P, et al. Phosphatidylinositol-4,5-bisphosphate hydrolysis directs actin remodeling during phagocytosis. J Cell Biol. 2005 Apr;169:139-149.

65. Jiang L, Ni Z, Wang L, Feng L, Liu B. Loss of the lac operon contributes to *Salmonella* invasion of epithelial cells through derepression of flagellarsynthesis. Curr Microbiol. 2015;70:315-323.

66. Pham OH, McSorley SJ. Protective host immune responses to *Salmonella* infection. Future Microbiol. 2015;10(1):101-110.

67. Levine MM, Farag TH. Invasive *Salmonella* infections and HIV in northern Tanzania. Clin Infect Dis. 2011;52(3):349-351.

68. Levine MM, Black RE, Lanata C. Precise estimation of the numbers of chronic carriers of *Salmonella typhi* in Santiago, Chile, an endemic area. J Infect Dis. 1982 Dec;146: 724-726.

69. Merselis JG Jr, Kaye D, Connolly CS, Hook EW. Quantitative bacteriology of the typhoid carrier state. Am J Trop Med Hyg. 1964 May;13:425-429.

70. Ivanoff B, Levine MM, Lambert PH. Vaccination against typhoid fever: present status. Bull World Health Organ. 1994;72(6):957-971.

71. Khatri NS, Maskey P, Poudel S, Jaiswal VK, Karkey A, et al. Gallbladder carriage of *Salmonella paratyphi* A may be an important factor in the increasing incidence of this infection in South Asia. Ann Intern Med. 2009;150:567-568.

72. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347:1770-1782.

73. Dongol S, Thompson CN, Clare S, Nga TVT, Duy PT, et al. The microbiological and clinical characteristics of invasive *Salmonella* in gallbladders from cholecystectomy patients in Kathmandu, Nepal. PLoS One. 2012 Oct;7:e47342.

74. Zaki SA, Karande S. Multidrug resistant typhoid fever: a review. J Infect Dev Ctries. 2011 May;5:324-337.

75. Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW. Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol Rev. 2009 Mar;33:376-393.

76. Chiou CS, Alam M, Kuo JC, Liu YY, Wang PJ. Chromosome-mediated multidrug resistance in *Salmonella enterica* serovar Typhi. Antimicrob Agents Chemother. 2015;59(1):721-723.

77. Kumar M, Dahiya S, Sharma P, Sharma S, Singh TP, et al. Structure based *in silico* analysis of quinolone resistance in clinical isolates of *Salmonella* Typhi from India. PLoS One. 2015;10:e0126560.

78. Emary K, Moore CE, Chanpheaktra N, An KP, Chheng K, et al. Enteric fever in Cambodian children is dominated by multidrug-resistant H58 *Salmonella enterica* serovar Typhi with intermediate susceptibility to ciprofloxacin. Trans R Soc Trop Med Hyg. 2012 Dec;106(12):718-724.

79. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive *Salmonella* disease. Vaccine. 2015 Jun;33:C21-C29.

80. Parry CM, Thuy CT, Dongol S, Karkey A, Vinh H,et al. Suitable disk antimicrobial susceptibility breakpoints defining *Salmonella enterica* serovar Typhi isolates with reduced susceptibility to fluoroquinolones. Antimicrob Agents Chemother. 2010;54:5201-5208.

81. Keddy KH, Smith AM, Sooka A, Ismail H, Oliver S. Fluoroquinolone-resistant typhoid, South Africa. Emerg Infect Dis. 2010;16:879-880.

82. Pfeifer Y, Matten J, Rabsch W. *Salmonella enterica* serovar Typhi with CTX-M beta-lactamase, Germany. Emerg Infect Dis. 2009 Sep;15:1533-1535.

83. Geetha VK, Yugendran T, Srinivasan R, Harish BN. Plasmid-mediated quinolone resistance in typhoidal *Salmonella*: a preliminary report from South India. Indian J Med Microbiol. 2014 Mar;32:31-34.

84. García-Fernández A, Gallina S, Owczarek S, Dionisi AM, Benedetti I, et al. Emergence of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi in Italy. PLoS One 2015; 10: e0132065.

85. Somily AM. An imported enteric fever caused by a quinolone-resistant *Salmonella typhi*. Ann Saudi Med. 2010 Jul;30:313-316.

86. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13:42-51.

87. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014 Aug;22:438-445.

88. Smith AM, Govender N, Keddy KH. Quinolone-resistant *Salmonella* Typhi in South Africa, 2003-2007. Epidemiol Infect. 2010 Jan;138:86-90.

89. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. Clin Infect Dis. 2010 Jan;50:241-246.

90. Rahman BA, Wasfy MO, Maksoud MA, Hanna N, Dueger E, et al. Multi-drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella enterica* serovar Typhi isolates from the Middle East and Central Asia. New Microbes New Infect. 2014 Jul;2(4):88-92.

91. Naheed A, Ram PK, Brooks WA, Hossain MA, Parsons MB, et al. Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. Int J Infect Dis. 2010 Sep;14:93-99.

92. Nagshetty K, Channappa ST, Gaddad SM. Antimicrobial susceptibility of *Salmonella* Typhi in India. J Infect Dev Ctries. 2010 Mar;4(2):70-73.

93. Hendriksen RS, Leekitcharoenphon P, Mikoleit M, Jensen JD, Kaas RS, et al. Genomic dissection of travel-associated extended-spectrum-beta-lactamase-producing *Salmonella enterica* serovar typhi isolates originating from the Philippines: a one-off occurrence or a threat to effective treatment of typhoid fever? J Clin Microbiol. 2015; 53:677-680.

94. Holt KE, Phan MD, Baker S, Duy PT, Nga TV, et al. Emergence of a globally dominant IncHI1 plasmid type associated with multiple drug resistant typhoid. PLoS Negl Trop Dis. 2012;5(7):e1245.

95. Wong VK, Baker S, Connor TR, Pickard D, Page AJ, et al. An extended genotyping framework for *Salmonella enterica* serovar Typhi, the cause of human typhoid. Nat Commun. 2016 Oct;5:12827.

96. Wong VK, Baker S, Pickard DJ, Parkhill J, Page AJ, et al. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of *Salmonella* Typhi identifies inter- and intracontinental transmission events. Nat Genet. 2015 Jun;47(6):632-639.

97. Baker S, Holt KE, Clements AC, Karkey A, Arjyal A, et al. Combined high-resolution genotyping and geospatial analysis reveals modes of endemic urban typhoid fever transmission. Open Biol. 2011 Oct;1:110008.

98. Holt KE, Dolecek C, Chau TT, Duy PT, La TTP, et al. Temporal fluctuation of multidrug resistant *Salmonella* Typhi haplotypes in the Mekong River delta region of Vietnam. PLoS Negl Trop Dis. 2011 Jan;5(1):e929.

99. Kariuki S, Revathi G, Kiiru J, Mengo DM, Mwituria J, et al. Typhoid in Kenya is associated with a dominant multidrug-resistant *Salmonella enterica* serovar Typhi haplotype that is also widespread in Southeast Asia. J Clin Microbiol. 2010 Jun;48(6):2171-2176.

100. Chiou CS, Lauderdale TL, Phung DC, Watanabe H, Kuo JC, et al. Antimicrobial resistance in *Salmonella enterica* serovar Typhi isolates from Bangladesh, Indonesia, Taiwan, and Vietnam. Antimicrob Agents Chemother. 2014 Nov;58(11):6501-6507.

101. Baltazar M, Ngandjio A, Holt KE, Lepillet E, Pardos de la Gandara M, et al. Multidrug-resistant *Salmonella enterica* serotype Typhi, Gulf of Guinea Region, Africa. Emerg Infect Dis. 2015 Apr;21(14): 655-659.

102. Steele AD, Hay Burgess DC, Diaz Z, Carey ME, Zaidi AKM. Challenges and opportunities for typhoid fever control: a call for coordinated action. Clin Infect Dis. 2016 Mar;62:S4-S8.

103. Feasey NA, Gaskell K, Wong V, Msefula C, Selemani G, et al. Rapid emergence of multidrug resistant H58-lineage *Salmonella* Typhi in Blantyre, Malawi. PLoS Negl Trop Dis. 2015 Apr;9:e0003748.

104. Pham Thanh D, Karkey A, Dongol S, Ho Thi N, Thompson CN, et al. A novel ciprofloxacin-resistant subclade of H58 *Salmonella* Typhi is associated with fluoroquinolone treatment failure. eLife. 2016;5:e14003.

105. Arjyal A, Basnyat B, Nhan HT, Koirala S, Giri A, et al. Gatifloxacin versus ceftriaxone for uncomplicated enteric fever in Nepal: an open-label, two-centre, randomised controlled trial. Lancet Infect Dis. 2016 May;16(5):535-545.

106. Baker S, Duy PT, Nga TV, Dung TT, Phat VV, et al. Fitness benefits in fluoroquinolone-resistant *Salmonella* Typhi in the absence of antimicrobial pressure. eLife. 2013 Dec;2:e01229.

107. Afzal A, Sarwar Y, Ali A, Haque A. Current status of fluoroquinolone and cephalosporin resistance in *Salmonella enterica* serovar Typhi isolates from Faisalabad, Pakistan. Pak J Med Sci. 2012;28:602-607.

108. Demczuk WHB, Finley R, Nadon C, Spencer A, Gilmour M, et al. Characterization of antimicrobial resistance, molecular and phage types of *Salmonella enterica* serovar Typhi isolations. Epidemiol Infect. 2010;138:1414-1426.

109. Chand HJ, Rijal KR, Neupane B, Sharma VK, Jha B. Re-emergence of susceptibility to conventional first line drugs in *Salmonella* isolates from enteric fever patients in Nepal. J Infect Dev Ctries. 2014;8(11):1483-1487.

110. Shrestha KL, Pant NA, Bhandari R, Khatri S, Shrestha B, et al. Re-emergence of the susceptibility of the *Salmonella* spp. isolated from blood samples to conventional first line antibiotics. Antimicrob Resist Infect Control. 2016 May;5:22.

111. Choudhary A, Gopalakrishnan R, Senthur NP, Ramasubramanian V, Abdul GK, et al. Antimicrobial susceptibility of *Salmonella enterica* serovers in a tertiary care hospital in southern India. Indian J Med Res. 2013 Apr;137:800-802.

112. Kanj SS, Kanafani ZA, Shehab M, Sidani N, Baban T, et al. Epidemiology, clinical manifestations, and molecular typing of *Salmonella typhi* isolated from patients with typhoid fever in Lebanon. J Epidemiol Global Health. 2015;5:159-165.

113. World Health Organization. Background document: The diagnosis, treatment and prevention of typhoid fever; 2003.

114. Breiman RF, Cosmos L, Njuguna H, Audi A, Olack B, et al. Population based incidence of typhoid fever in an urban informal settlement and a rural area in Kenya: implication for typhoid vaccine use in Africa. PLoS One. 2012;7:e29119.

115. Shahunja KM, Leung DT, Ahmed T, Bardhan PK, Ahmed D, et al. Factors associated with non-typhoidal *Salmonella* bacteremia versus typhoidal *Salmonella* bacteremia in patients presenting for care in an urban diarrheal disease hospital in Bangladesh. PLoS Negl Trop Dis. 2015;9(9):e0004066.

116. Wain J, Hendriksen RS, Mikoleit M, Keddy KH,Ochiai RL. Typhoid fever. Lancet. 2015;385:1136-1145.

117. Irfan S, Khan E, Jabeen K, Bhawan P, Hopkins KL, Day M, et al. Clinical isolates of *Salmonella enterica* serovar Agona producing NDM-1 metallo-β-lactamase: first report from Pakistan. J Clin Microbiol. 2015 Jan;53:346-348.

118. Levine MM, Sztein MB. Vaccine development strategies for improving immunization: the role of modern immunology. Nat Immunol. 2004 May;5(5):460-464.

119. Centers for Disease Control and Prevention. Typhoid immunization – recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1994;43:1-7.

120. Date KA, Bentsi-Enchill AD, Fox KK, Abeysinghe N, Mintz ED, Khan MI, et al. Typhoid fever surveillance and vaccine use – South-East Asia and Western pacific regions, 2009-2013. MMWR Morb Mortal Wkly Rep. 2014 Oct;63:855-860.

121. Hardy A. Methods of outbreak investigation in the "era of bacteriology" 1880-1920. Soz Praventivmed. 2001;46(6):355-360.

122. Wong KH, Feeley JC, Northrup RS, Forlines ME. Vi antigen from *Salmonella typhosa* and immunity against typhoid fever. I. Isolation and immunologic properties in animals. Infect Immun. 1974 Feb;9:348-353.

123. Wong KH, Feeley JC. Isolation of Vi antigen and a simple method for its measurement. Appl Microbiol. 1972 Oct;24:628-633.

124. Steinberg EB, Bishop R, Haber P, Dempsey AF, Hoekstra RM, et al. Typhoid fever in travelers: who should be targeted for prevention? Clin Infect Dis. 2004;39:186-191.

125. Klugman KP, Koornhof HJ, Robbins JB, Le Cam NN. Immunogenicity, efficacy and serological correlate of protection of *Salmonella typhi* Vi capsular polysaccharide vaccine three years after immunization. Vaccine. 1996 Apr;14:435-438.

126. Levine MM, Tacket CO, Sztein MB. Host-*Salmonella* interaction: human trials. Microbes Infect. 2001 Nov;3:1271.

127. Kantele A, Arvilommi H, Kantele JM, Rintala L, Makela PH. Comparison of the human immune response to live oral, killed oral or killed parenteral *Salmonella typhi* Ty21a vaccines. Microb Pathog. 1991 Feb;10(2):117-126.

128. Salerno-Goncalves R, Pasetti MF, Sztein MB. Characterization of $CD8^+$ effector T cell responses in volunteers immunized with *Salmonella enterica* serovar Typhi strain Ty21a typhoid vaccine. J Immunol. 2002. Aug;169(4):2196-2203.

129. Lundin BS, Johansson C, Svennerholm AM. Oral immunization with a *Salmonella enterica* serovar typhi vaccine induces specific circulating mucosa-homing $CD4^+$ and $CD8^+$ T cells in humans. Infect Immun. 2002;70:5622-5627.

130. Hohmann EL, Oletta CA, Miller SI. Evaluation of a phoP/phoQ-deleted, aroA-deleted live oral *Salmonella typhi* vaccine strain in human volunteers. Vaccine. 1996 Jan;14:19-24.

131. Salerno-Goncalves R, Wahid R, Sztein MB. Immunization of volunteers with *Salmonella enterica* serovar Typhi strain Ty21a elicits the oligoclonal expansion of $CD8^+$ T cells with predominant Vβ repertoires. Infect Immun. 2005 Jun;73(6):3521-3530.

132. Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, et al. Duration of efficacy of Ty21a, attenuated *Salmonella typhi* live oral vaccine. Vaccine. 1999;17:S22-27.

133. Jackson BR, Iqbal S, Mahon B. Updated recommendations for the use of typhoid vaccine – Advisory Committee on Immunization Practices, United States, 2015. MMWR Morb Mortal Wkly Rep. 2015 Mar;64;305-308.

134. Ochiai RL, Acosta CJ, Agtini M, Bhattacharya SK, Bhutta ZA, et al. The use of typhoid vaccines in Asia: the DOMI experience. Clin Infect Dis. 2007 Jul;45:S34-S38.

135. Simanjuntak CH, Paleologo FP, Punjabi NH, Darmowigoto R, Soeprawoto, et al. Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine. Lancet. 1991;338:1055-1059.

136. Levine MM, Ferreccio C, Cryz S, Ortiz E. Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in randomised controlled field trial. Lancet. 1990 Oct;336:891-894.

137. Fraser A, Goldberg E, Acosta CJ, Paul M, Leibovici L. Typhoid fever vaccines: systematic review and meta-analysis of randomized controlled trials. Vaccine. 2007;45:7848-7857.

138. Ferreira RBR, Antunes LCM, Finlay BB. Should the human microbiome be considered when developing vaccines? PLoS Pathog. 2010;6:e1001190.

139. McArthur MA, Szetein MB. Heterogeneity of multifunctional IL-17A producing *S. typhi*-specific $CD8^+$ T cells in volunteers following Ty21a typhoid immunization. PLoS One. 2012;7:e38408.

140. Pakkanen SH, Kantele JM, Savolainen LE, Rombol L, Kantele A. Specific and cross-reactive immune response to oral *Salmonella* Typhi Ty21a and parenteral Vi capsular polysaccharide typhoid vaccines administered concomitantly. Vaccine. 2015;33:451-458.

141. Szetein MB. Cell-mediated immunity and antibody responses elicited by attenuated *Salmonella enterica* serovar Typhi strains used as live oral vaccines in humans. Clin Infect Dis. 2007;45 suppl 1:S15-19.

142. Mastroeni P, Chabalgoity JA, Dunstan SJ, Maskell DJ, Dougan G. *Salmonella*: immune responses and vaccines. Vet J. 2001;161:132-164.

143. Sztein MB, Wasserman SS, Tacket CO, Edelman R, Hone D, et al. Cytokine production patterns and lymphoproliferative responses in volunteers orally immunized with attenuated vaccine strains of *Salmonella typhi*. J Infect Dis. 1994;170:1508-1517.

144. Wahid R, Salerno- Goncalves R, Tacket CO, Levine MM, Sztein MB. Cell-mediated immune responses in humans after immunization with one or two doses of oral live attenuated typhoid vaccine CVD 909. Vaccine. 2007;25:1416-1425.

145. Salerno-Gonçalves R, Wyant TL, Pasetti MF, Fernandez-Viña M, Tacket CO, et al. Concomitant induction of $CD4^+$ and $CD8^+$ T cell responses in volunteers immunized with *Salmonella enterica* serovar Typhi strain CVD 908-htrA. J Immunol. 2003;170:2734-2741.

146. Tacket CO, Sztein MB, Wasserman SS, Losonsky G, Kotloff KL, et al. Phase 2 clinical trial of attenuated *Salmonella enterica* serovar Typhi oral live vector vaccine CVD 908-htrA in U.S. volunteers. Infect Immun. 2000 Mar;68:1196-1201.

147. Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, Nataro JP, et al. Safety of live oral *Salmonella typhi* vaccine strains with deletions in *htrA* and *aroC aroD* and immune response in humans. Infect Immun. 1997;65:452-456.

148. Gonzalez C, Hone D, Noriega FR, Tacket CO, Davis JR, et al. *Salmonella typhi* vaccine strain CVD 908 expressing the circumsporozoite protein of *Plasmodium falciparum*: strain construction and safety and immunogenicity in humans. J Infect Dis. 1994;169:927-931.

149. Tacket CO, Pasetti MF, Sztein MB, Livio S, Levine MM. Immune responses to an oral typhoid vaccine strain that is modified to constitutively express Vi capsular polysaccharide. J Infect Dis. 2004;190:565-570.

150. Robbins JD, Robbins JB. Reexamination of the protective role of the capsular polysaccharide (Vi antigen) of *Salmonella typhi*. J Infect Dis. 1984;150:436-449.

151. Acharya IL, Lowe CU, Thapa R, Gurubacharya VL, Shrestha MB, et al. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi*. A preliminary report. N Engl J Med. 1987;317:1101-1104.

152. Holmgren J, Syennerholm AM. Vaccines against mucosal infections. Curr Opin Immunol. 2012;24:343-353.

153. Yang HH, Kilgore PE, Yang LH, Park JK, Pan YF, et al. An outbreak of typhoid fever, Xing-An County, People's Republic of China, 1999: estimation of the field effectiveness of Vi polysaccharide typhoid vaccine. J Infect Dis. 2001;183:1775-1780.

154. Yang HH, Wu CG, Xie GZ, Gu QW, Wang BR, et al. Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. Bull World Health Organ. 2001;79:625-631.

155. Acosta CJ, Hong-Hui Y, Ning W, Qion G, Qun D, et al. Efficacy of a locally produced, Chinese Vi polysaccharide typhoid fever vaccine during six years of follow-up. Vaccine. 2005;23:5618-5623.

156. Ochiai RL, Khan MI, Soofi SB, Sur D, Kanungo S, et al. Immune responses to Vi capsular polysaccharide yyphoid vaccine in children 2 to 16 years old in Karachi, Pakistan, and Kolkata, India. Clin Vaccine Immunol. 2014;21:661-666.

157. World Health Organization. Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. WHO Technical Report Series No. 987;2014. http://www.who.int/biologicals/areas/vaccines/TRS_987_Annex3.pdf?ua=1

158. Zhou WZ, Koo HW, Wang XY, Zhang J, Park JK, et al. Revaccination with locally-produced vi typhoid polysaccharide vaccine among Chinese school-aged children: safety and immunogenicity findings. Pediatr Infect Dis J. 2007;26:1001-1005.

159. Thiem VD, Lin FY, Canh DG, Son NH, Anh DD, et al. The Vi conjugate typhoid vaccine is safe, elicits protective levels of IgG anti-Vi, and is compatible with routine infant vaccines. Clin Vaccine Immunol. 2011;18:730-735.

160. Plotkin SA, Bouveret-Le Cam N. A new typhoid vaccine composed of the Vi capsular polysaccharide. Arch Intern Med. 1995;155:2293-2299.

161. Kantele A, Pakkanen SH, Karttunen R, Kantele JM. Head-to-head comparison of humoral immune responses to Vi capsular polysaccharide and *Salmonella* Typhi Ty21a typhoid vaccines – a randomized trial. PLoS One. 2013;8:e60583.

162. vanDamme P, Kafeja F, Anemona A, Basile V, Hilbert AK, et al. Safety, immunogenicity and dose ranging of a new Vi-CRM_{197} conjugate vaccine against typhoid fever: randomized clinical testing in healthy adults. PLoS One. 2011;6:e25398.

163. Lin FYC, Ho VA, Khiem HB, Trach DD, Bay PV, et al. The efficacy of a *Salmonella typhi* Vi conjugate vaccine in two-to-five-year-old children. N Engl J Med. 2001;344:1263-1269.

164. Canh DG, Lin FYC, Thiem VD, Trach DD, Trong ND, et al. Effect of dosage on immunogenicity of a Vi conjugate vaccine injected twice into 2-to 5-year-old Vietnamese children. Infect Immun. 2004;72:6586-6588.

165. Shi H, Santander J, Brenneman KE, Wanda SY, Wang S, et al. Live recombinant *Salmonella* Typhi vaccines constructed to investigate the role of *rpoS* in eliciting immunity to a heterologous antigen. PLoS One. 2010;5:e11142.

166. Szu SC. Development of Vi conjugate – a new generation of typhoid vaccine. Expert Rev Vaccines. 2013;12:1273-1286.