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 cause 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 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-Kaufmann-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 2.52 to 5.03 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 (SPIs). 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 SPIs 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 SPIs; four are specific to *S. typhi* (SPI-7, 15, 17 and 18) and only one (SPI-14) for *S. typhimurium* [25]. The *viA* gene which is located in SPI-1 is a regulator of the Vi polysaccharide antigen [26]. 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 (SPI-1 and SPI-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 flagellum motility regulator PhDC [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 *blo* in the transfer and spread of antimicrobial resistance has been well described [40].

**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 actinomycetemcomitans*, *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 AB5-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 A5B5 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 *PltA* 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*, *TxsA*, *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 *A5*...
structure requires a reducing atmosphere to dissociate the two
subunits, PLT and GTb; (iv) the receptors to bind to the host cell
utilize PBtR rather than GTa and Dct 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, diarrhoea or
constipation, headache, relative bradycardia and reduced level of
consciousness. Potential complications include intestinal perforation
and membrane trafficking of the reticuloendothelial system [67].

Rho-family GTPases and phosphoinositides are involved in the cell signal
transduction pathway, actin remodeling and membrane trafficking
process of phagocytosis in non-phagocytic cells such as epithelial
cells in the colon and respiratory tract [68-69]. 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 H1, 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, SG11 is a
*Salmonella* genomic island, identified for the first time in multidrug-resistant (MDR) *S. typhi*. For example, SG11-carrying *S. typhi*
possessing seven resistance genes (*bla*, *catA1*, *strA*, *strB*, *sul1*, *sul2*
and *draF*) 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*) and 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 *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qnrD*, plasmid *qepA*, *qepAB* 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,
sulphonamides and trimethoprim, and which were thereby classified
as being MDR. *S. typhi* haptoglobin H58, containing IncH1 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 IncH1 ST6 plasmids, which has been the most prevalent haptolype (87%) in Kenya since the early 2000s [98,99].
Likewise, from 1995 onwards 98% of MDR *S. typhi* isolates were
of the same H58 haptolype, 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
haptotypes were recovered but rather MDR quinolone-susceptible
isolates carried a 190-kb IncH1 pST2 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 Melong river delta of Vietnam was caused exclusively by a H58 nalidixic acid-resistant haptolype 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 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 become overrepresented.

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 blaCTX-M-15 and qnrB2, which reduces 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 Vivifluc by Berne 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- and aceton-killed bovine killed 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 the liquid and enteric coated vaccines were 53% and 44%, 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 socio-economic status, genetic make-up, nutritional status, 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 cytokine such as interferon (IFN)-γ 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-Atr 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-CRM197, 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 PsP 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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