**Review Article**

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# Typhoid fever & vaccine development: a partially answered question

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**Typhoid fever is a systemic disease caused by the human specific Gram-negative pathogen *Salmonella enterica* serovar Typhi (*S.* Typhi). The extra-intestinal infections caused by *Salmonella* are very fatal. The incidence of typhoid fever remains very high in impoverished areas and the emergence of multidrug resistance has made the situation worse. To combat and to reduce the morbidity and mortality caused by typhoid fever, many preventive measures and strategies have been employed, the most important being vaccination. In recent years, many *Salmonella* vaccines have been developed including live attenuated as well as DNA vaccines and their clinical trials have shown encouraging results. But with the increasing antibiotic resistance, the development of potent vaccine candidate for typhoid fever is a need of the hour. This review discusses the latest trends in the typhoid vaccine development and the clinical trials which are underway.**

**Key words** DNA vaccine - immunity - *Salmonella* - typhoid fever - vaccination

## Introduction

Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, multicellular parasites or fungi, protozoa and aberrant proteins namely prions. These diseases are known to be contagious. Evidence of infective diseases has been reported in history[1]. There are many infectious diseases which are more geographically localized, but still create worldwide concern, *e.g*., haemorrhagic fever, Nipah virus, monkeypox, *etc*. Today, in spite of having increased knowledge about causative agents of many infectious diseases, we are far behind from controlling many of these and in the development of an efficient vaccine. Typhoid fever is one such disease, which is caused by *Salmonella enterica* serovar Typhi. Though salmonellosis is rare in developed and industrialized countries, it still remains a serious problem in most of the developing countries especially Southeast Asian countries, Africa and Latin America.

*S. enterica* is a Gram-negative intracellular pathogenic bacterium which infects humans and many warm blooded animals. *S. enterica* includes 2500 serovars most of which have been described as human pathogens but only a few are of public health importance[2,3]. *Salmonella* can infect a wide range of host including reptiles, birds and mammals. However, some serovars are highly specific *viz*. *S.* Typhi or *S.* Paratyphi[3].

## Disease impact of typhoid fever

Some of the *Salmonella* serovars are known to cause osteomyelitis, splenic abscess and septicemia[4].

*S.* Typhi or *S.* Paratyphi infect human and cause typhoid and paratyphoid fever, respectively. Transmission of the disease occurs through faecal-oral route, upon ingestion of contaminated water and food and inadequate sanitation, consuming raw milk products, flavored drinks and ice-creams. This disease can also spread through consumption of raw fruits and vegetables grown in fields irrigated with sewage water and fertilizer[5,6]. The incubation period of the disease is usually 10-14 days and varies considerably from 8-15 days, but may be as short as 5 days and as long as 30 or 35 days depending upon the inoculum size and the state of host defenses. Occurrence of the disease has to be confirmed by the presence of the pathogen either *S.* Typhi or *S.* Paratyphi in patient, which requires isolation of the bacteria from blood, stool or bone marrow. The sensitivity of the test decreases with increased duration of fever. Another method is Widal test, which identifies the presence of antibodies against *Salmonella* specific O (somatic) and H (flagellar) antigens in the serum which appear only in the 2nd week after the disease onset. One of the recent diagnostic techniques developed in our laboratory is a PCR based method which utilizes specific primer designed against a region unique to *S.* Typhi and *S.* Paratyphi A. This method can detect very small number of bacteria within 4-5 days of onset of infection[7]. This method is more sensitive than Widal test and can also differentiate between *S.* Typhi CT18, *S.* Typhi Ty2 and *S.* Paratyphi A.

Most commonly used antibiotics for the treatment of typhoid fever are fluoroquinolone such as ciprofloxacin, ofloxacin and pefloxacin and third-generation cephalosporins such as ceftriaxone or cefotaxime. Antibiotic treatments are often ineffective in carriers with gallstones. In these carriers, *Salmonella* are resistant to bile[8] forming biofilm on the gallbladder conferring antimicrobial resistance to bacteria[9]. Emergence of multidrug resistance (MDR) in *Salmonella* has made treatment of the disease more difficult and complicated. In 1948, chloramphenicol was first reported for the treatment of typhoid fever[10]. In 1972, chloramphenicol resistance was identified as a major problem in the treatment of disease during an outbreak in Mexico, India, Vietnam, Thailand, Korea and Peru[11]. The resistance to chloramphenicol was associated with high-molecular-weight, self-transferable, *Inc*HI plasmids which encode the resistance genes; but strains of *S.* Typhi with such plasmid mediated MDR are not observed in Latin America[12,13]. In the 1970s and 1980s, use of antibiotics like fluoroquinolones such as ciprofloxacin and ofloxacin had become widespread especially in countries where MDR was a problem. In 2005, the existence of MDR strains of *S.* Typhi was noticed in patients in Lagos, Nigeria[14], ciprofloxacin resistance in Karachi and in 2007 in India, MDR strains of *S.* Typhi were reported[15]. MDR strains of *S.* Typhimurium have become a major cause of salmonellosis worldwide *e.g.* MDR definitive phage type 104 (DT104), which was found to be resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline[16,17].

MDR is also mediated by another plasmid *p*HCM1, in bacteria[18]. The situation has further worsened because some variants of *Salmonella* have developed MDR as an integral part of their genome and therefore, are likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, making the pathogen more virulent and potent. One such example is the chromosomally encoded gene complex known as the *Salmonella* genomic island 1 in *S.* Typhimurium[19], which can be transferred to other *Salmonella* serotypes[20]. Thus, the emergence and global spread of antimicrobial resistant *Salmonella* strains have necessitated to understand in depth the mechanism of pathogenesis so as to find new drug target for the MDR strains of the pathogen and develop a vaccine as a prophylactic strategy. During its intracellular life in macrophages, *Salmonella* induces a variety of regulatory systems which are responsible for its survival inside the host[21]. *Salmonella* has evolved different strategies to evade the host immune response. One of the strategies is modification of lipopolysaccharides (LPS) which is normally recognized by TLR-4 triggering downstream signaling cascade to evoke host immune response[22,23]. This membrane remodelling blocks recognition by host TLR-4 and also increases resistance of bacteria against host antimicrobial peptides. *Salmonella* also prevents the fusion of *Salmonella* containing vacuole with lysosome and vesicles containing reactive oxygen and nitrogen species[24]. *Salmonella* inhibits antigen presentation via dendritic cells[25,26]. There are many regulators in *Salmonella* like PhoP/Q, PmrA/B, OmpR/EnvZ, two component system (TCS), sigma factor RpoS and RpoE, which are responsible for regulating such phenotypes. PhoP/Q TCS is required for antimicrobial peptide (AMP) resistance, virulence and survival of *Salmonella* within macrophages[27]. PmrA/B TCS was described for the first time in 1993[28] and was later associated with resistance against AMPs like polymyxin B, Cationic antimicrobial protein 37

and Cationic antimicrobial protein 57 (BPI)[29]. Some of these regulated genes are involved in LPS modification as well as AMPs resistance. PmrA/B and PhoP/Q, regulate genes in mouse model especially in GI tract and in spleen[30].

**Vaccine status for *Salmonella* pathogenesis/typhoid fever**

Typhoid fever caused by *S.* Typhi or *S.* Paratyphi is a major health problem with global incidence of 21 million cases and 200,000 (1-4% death worldwide) deaths per year[31]. *Salmonella* has become a major threat to the society due to the disease severity, recurrence of disease through carrier state, emergence of multidrug resistance and its use as a potential candidate in bioterrorism[32]. This demands for an effective prophylactic measures. In 2000 and 2008, WHO has explained the importance of vaccine against typhoid fever[33,34]. There have been many efforts done by different groups of scientists to develop an effective vaccine against *Salmonella.* But at present, only two licensed vaccine for typhoid fever - a subunit (Vi PS) and a live attenuated *S.* Typhi strain (Ty21a) are commercially available. Continuous efforts are being undertaken to develop typhoid vaccine with the advancement of Vi polysaccharide conjugate vaccine and live attenuated *Salmonella* strain to attain higher antibody titres and increased immunogenicity[35]. Murine model of typhoid fever (BALB/c mice infection with *S.* Typhimurium) is used initially to test the efficiency and potency of the vaccine. Once the vaccine is found to be safe, it will be underaken for clinical/field trial in humans.

**Inactivated whole-cell typhoid vaccine**

In 1896, heat killed phenol preserved and acetone killed lyophilized injectable whole cell *S.* Typhi vaccine was generated and used in England and Germany. The efficacy of this vaccine was assessed in a trial in 1960 in Yugoslavia, USSR, Poland, and Guyana. This vaccine is still in use in a few countries but most of the countries have withdrawn the usage of this vaccine due to the side effects. The acetone killed vaccine was more superior to heat-phenol killed vaccine. It was due to the preservation of Vi-polysaccharide in acetone killed vaccine[36,37]. However, inactivated whole-cell vaccine causes local inflammation, pain, systemic fever, malaise and disease like symptoms in 9-34 per cent of the recipients. Thus, whole cell inactivated vaccine was not considered suitable for public use and in spite of being licensed it is no longer available in the market[38,39]. Unfortunately for paratyphoid fever, there is no licensed vaccine till date. Increasing emergence of multidrug resistance strains of *Salmonella* has further complicated the situation of the disease and its treatment with existing antibiotics.

**Vi-polysaccharide (Vi-PS) vaccine**

Felix and co-workers showed the presence of serological response against Vi-polysaccharide in typhoid fever[40,41]. Considering this and the ability of acetone killed whole-cell vaccine to be more effective due to presence of Vi-polysaccharide[36,37], it was considered as a potential candidate for vaccine development. In 1986, Robbins and Robbins at NIH developed an injectable subunit vaccine Vi-polysaccharide vaccine (sold as Typhim Vi by Sanofi Pasteur and Typherix by GlaxoSmithKline). The vaccine was licensed to Sanofi-Pasteur in 1994 (Typhim TM, Sanofi-Pasteur; TypbarTM, Bharar). Vi polysaccharide, a linear homopolymer of galacturonic acid is purified from the bacteria by treatment with detergent Cetavlon. During initial trial, this vaccine gave 75 per cent protection during 20 months surveillance in 5-44 yr age group in Nepal. In South Africa, among the age group of 5-16 yr, 64 per cent protection was observed after 21 months and after 3 yr the protection was only 55 per cent[42,43]. In China, the protection among 5-9 yr old children was 69 per cent[44]. This vaccine has certain drawbacks being non-immunogenic in children below 2 yr of age and unable to induce booster effect. Most importantly many of the *S.* Typhi strains are negative for Vi polysaccharide or lose their Vi-antigen[45, 46] and in such cases Vi-PS vaccine will not be able to protect the patient. The recent data of the community vaccination in high-incidence areas of Kolkata, Karachi, and North Jakarta showed the cost effectiveness of Vi-polysaccharide vaccine in children[47].

**The live attenuated Ty21a vaccine**

Due to the side effects and low effectiveness of the killed whole cell vaccine a need for a more competent vaccine candidate emerged. With the knowledge that live attenuated strain elicits more immune response, attenuated *Salmonella* strains were considered for vaccine development. Ty21a was the first live oral attenuated *Salmonella* vaccine (sold as Vivotif by Berna Biotech, now crucell and was developed in Switzerland by chemical mutagenesis of wild-type *S.* Typhi strain Ty2[38, 48]. This strain lacks both functional galactose-epimerase (*galE*) gene and the Vi antigen and is highly attenuated. The Ty21a vaccine is licensed in 56 countries of Asia, Africa, USA and Europe[1].

This vaccine is available in both liquid as well enteric coated capsule forms. Clinical trials in Alexandria, Egypt, with the liquid form of vaccine showed 96 per cent protection for 3 yr and in Santiago, Chile, it was found to be 67 per cent for 3 yr and 62 per cent over 7 yr[49]. Ty21a, was also shown to provide 42-56 per cent protection against paratyphoid caused by *S.* Paratyphi, in Chile[48]. It is also hypothesized that Ty21a can give rise to herd-immunity effect[38]. Despite an adequate immune response and efficacy against typhoid fever, Ty21a has certain drawbacks. To obtain sufficient immunity, high numbers ($10^9$) of bacteria are required for oral dose; its use is recommended for children only above 5-6 yr of age. This vaccine is highly acid-labile and hence stomach acidity has to be either neutralized or bypassed when Ty21a is to be fed orally.

**Other non-commercialized vaccines against typhoid fever**

Several live attenuated *S.* Typhi strains have been developed for oral vaccination against typhoid fever. Many of the developed strains have been used in humans to check the efficacy and immunity against the pathogen. 541Ty (Vi+) and 543Ty (Vi-) were developed by Stocker and co-worker by transducing deletion in *aroA, purA* and *hisG*[51, 52]. 543Ty is a spontaneous mutant of 541Ty that lacks Vi-polysaccharide antigen. These strains are unable to maintain growth in mammalian tissue. 541Ty and 543Ty induced good immune response in the Phase I clinical trial[53]. Another attenuated strain, CVD 908 with mutation in *aroC* and *aroD* was developed[54]. A further mutation in the *htrA* locus, gave rise to the CVD908-*htrA*[55] vaccine strain with multiple deletions in *aroC*/*aroD*/*htrA*. When compared with the usage of existing vaccine Ty21a, single-dose CVD 908-*htrA* stimulated vigorous mucosal, humoral, and cellular immune responses that equaled or surpassed those measured after multiple doses of Ty21a. In 1992, Tacket and colleagues developed vaccine candidate χ3927 which is mutated in *cya* and *crp.* Along with other two CVD 906 (ISP1820 Δ*aroC* Δ *aroD*), CVD 908 (Ty2 Δ *aroC* Δ *aroD*) mutated vaccine strains, χ3927 vaccine were able to induce LPS specific antibody response when used in the volunteers[56].

Till date there was no effective vaccine that could confer protection to 2-5 yr old children. *Salmonella* Typhi Vi O-Acetyl Pectin-rEPA conjugate vaccine, a modified conjugate vaccine where Vi-PS is conjugated to a non-toxic recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA) has been used in clinical trial among the children and infants of age under 2 yr[18]. The trial reports an efficacy of 90 per cent in 2-5 yr old children.

The live attenuated candidate vaccine Ty800, a deletion mutant of the global regulator *phoP/phoQ* of Ty2, is developed by AVANT Immunotherapeutics[57,58]. It has passed Phase II trials and Ty800 has been shown to stimulate vigorous IgA and anti-O serum antibody responses in volunteers. *Salmonella* live attenuated vaccine candidate M01ZH09/ZH09 against *S.* Typhimurium/Typhi was developed with the deletion in the SPI gene (Ty2 *aroC* and *ssa*V) and underwent Phase II trial[59]. This vaccine strain lacks SPI-2 structural protein SsaV, required for secretion of bacterial effectors proteins. The mutation will hinder the SPI-2 function and prevent the systemic spread. Another mutation in *aroC*, aromatic mutation deprives the bacterium of essential nutrients it must obtain from the mammalian host. This double mutation vaccine strain was shown to be immunogenic and well-tolerated when given as a single dose. The study was conducted till 6 months after vaccination and volunteers did not show any bacteremia or prolonged shedding of the vaccine strain, which is an important safety advantage of vaccination. None of the new oral vaccine candidates (such as CVD 908, CVD 908-*htrA,* Ty800, and MO1ZH09), were consistent in their ability to stimulate serum Vi antibody. Levine and his group proposed that, if the expression of Vi is rendered to be constitutive in CVD 908-*htrA*, the strain might elicit the serum and mucosal anti-Vi antibody in addition to whole cell antigen response. They replaced the highly regulated P*tviA* in CVD 908-*htrA* with the strong constitutive promoter P*tac*, resulting in a strain "CVD 909" which constitutively expresses the Vi antigen[60,61]. This strain was the first vaccine candidate to elicit serum Vi antibodies and was able to significantly reduce the mortality of mice even with single immunization.

In 2007, a live attenuated vaccine against typhoid fever DV-STM-07 was developed in our laboratory, which has been shown to be potent in murine model of salmonellosis[62]. This is a multiple deletion mutant where the LPS modification and AMP resistance genes (*pmrG, pmr HIFJKLM* and *pmrD*) have been targeted and used as live vaccine. This vaccine was shown to provide immunity and protection at very low dose as well as with single dose, and was able to protect the pregnant mice and the foetus from lethal *Salmonella* infection. During the same year, CKS362, a killed but metabolically active (KBMA) *S.* Typhimurium strain was developed as a *Salmonella* vaccine.

**Table** Clinical trials of typhoid vaccine

| Vaccine | Mutation/Modification | Clinical trial (yr) | Age of volunteer (yr) | Dose | Efficacy/efficiency |
|---|---|---|---|---|---|
| Inactivated whole-cell typhoid vaccine (K and L vaccine)[35] | heat-phenol-inactivated (L) or acetone-inactivated (K) | In use since 1896. Field trials (1950s and 1960s), licensed for ≤6 yr age. not in use except few countries (Thailand) | 2 to 50 (mostly children & youngsters) | $10^8$ | 51-67% (L) 75-94% (K) |
| Ty21a (Vivotif Berna™ vaccine)[41] | galE and Vi Antigen | Field trials (1978-1981), licensed | 6 to 7 | $10^9$ | 0-100% (depending on immunizaion schedule and vaccine formulation) |
| 54l Ty[57] | *aroA*, *purA*, & *hisG* of *S.* Typhi strain CDC1O-80 (phage type A) | Phase 1 (1987) | 18 to 33 | $10^8$ $10^9$or $10^{10}$ | - |
| 543Ty[57] | *Vi*, *aroA*, *purA*, & *hisG* of *S.* Typhi strain CDC1O-80 (phage type A) | Phase 1 (1987) | 18 to 33 | $10^8$ $10^9$or $10^{10}$ | - |
| CVD 906[58] | *aroC* and *aroD* derivative of virulent *S.* Typhi strain ISP1820 | Phase 1 (1991) | 18 to 33 | 5 × 103 | 25% (expected protection) |
| CVD 908[49] | *aroC* and *aroD* derivative of virulent *S.* Typhi strain Ty2 | Phase 1 (1991) | 18 to 35 | $5 \times 10^4$/ $10^5$ | - |
| Typhim Vi/ Typherix[56,59] | purified Vi antigen | licensed (1994) | 5 to 44 | 25µg | Typhim Vi - 55-74%, Typherix - 80% |
| CVD 908-htrA[46,60] | *aroC*, *aroD* and *htrA* derivative of virulent *S.* Typhi strain Ty2 | Phase 1(1996), phase 2 (2000) | 18 to 40 | $5\times10^6$ and $5\times10^9$; $5\times10^7$ $4.5\times10^8$ | - |
| CVD 909[46,48] | CVD 908-htrA constitutively expressing Vi | trial in mice (2000), Phase 1 (2006) | – | $2.5 \times 10^9$ | - |
| Salmonella Typhi Vi O-Acetyl Pectin-rEPA Conjugate[61] | Vi bound to recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA) | Phase 2 (1991-2001) | 2 to 5, 5 to 14, adults | 22.5 µg of Vi and 22 µg of rEPA | 90% in 2-5 yr olds for 47 months |
| vax-TyVi®[62] | purified Vi antigen | trial (2002), licensed | 9 to 13 | 25 µg | 62.5–96.3% |
| Ty800[43] | *phoP*/*phoQ* of *S.* Typhi Ty2 | Phase 2 (2007) | 18 to 45 | $10^7$, $10^8$, $10^9$ or $10^{10}$ | - |
| MO1ZH09[63] | *aroC* & *ssaV* in *S.*Typhi | Phase 2 (2007-2009) | 2 to 17 | $5\times 10^9$ | - |
| DV-STM-07[50] | *pmrG*, *pmrH-M* and *pmrD* | mice study (2007) | | | - |
| DNA vaccine[55] | *sopB* | mice study (2009) | | | - |
| Vi-CRM197[64] glycoconjugated vaccine | chemical conjugation of the Vi polysaccharide of *S.* Typhi and the O polysaccharide of *S.* Paratyphi A to the carrier protein CRM197 | Phase 1 (2010-ongoing), phase 2 (planned to start in December 2010) | Adult (18-40 yr) | 1.25, 5 or 12.5 µg | - |

Superscript numerals denote reference numbers

The safety profile of KBMA strain was speculated to be similar to that of killed micro-organisms. *Salmonella* KBMA strain was derived from Δ*phoP/phoQ*Δ*aroA*[63]. This mutant vaccine strain was further made devoid of *uvrAB*, genes involved in DNA repair mechanism. Photochemical treatment of bacteria mutant in DNA excision repair (*ΔuvrAB*) renders the organisms "killed but metabolically active". This strain will not be able to replicate after treatment with UVA light. Also, these bacteria cannot synthesize psoralen which makes adduct with DNA in the presence of UVA and will be eliminated during the DNA repair by *uvrAB*. KBMA CKS362 vaccine strain was markedly less reactive, and stimulated a humoral immune response equivalent to its live counterpart and was more attenuated and immunogenic than live strain.

In 2002, a *Salmonella* vaccine named complex vaccine (CV) was designed which constituted of flagellin and polysome purified from *Salmonella* Typhimurium LT2[64]. Flagellin can induce T cell specific immunity against *Salmonella* and polysome can induce mucosal immunity and act as Th1 specific mucosal adjuvant. Flagellin and polysome were combined in 1:1 ratio by fomaldehyde condensation to develop CV. Oral vaccination using CV along with cholera toxin (CT), was able to provide complete protection unlike single antigen vaccination[64].

The typhoid vaccines designed till date give best protection to children above 2 yr of age. Considering the fact that young age group (1-5 yr) is more susceptible to *Salmonella* infection[65-68], Novartis Vaccines Institute for Global Health developed a Vi-CRM197 vaccine to ensure improved protection to this age group. Human clinical trials have been planned with adults (18-45 yr), children (24-59 months), older infants (9-12 months) and infants (6 wk of age) in December 2010 (Table). The vaccine was prepared by independent chemical conjugation of Vi-PS of *S*. Typhi and O polysaccharide of *S*. Paratyphi A to CRM197, a non-toxic mutant of diphtheria toxin[69]. The vaccine was well tolerated in animal model and found to induce a better antibody response compared to unconjugated Vi-PS[67]. It is expected that the vaccine will provide protection to infants and young children.

## DNA vaccine for typhoid fever

Epitope selection based vaccine development has been tried for long. The designing of the small DNA sequence based vaccine for many infectious diseases such as typhoid fever seems to be a promising area of research. Many efforts are being carried out in this direction. In 2004, a report stated that certain antigens of *Salmonella* showing high expression *in vivo* were preferentially recognized by CD4 T cells. Five peptides (Mig-14, IicA, SseB, SsaJ, or SifB) of *Salmonella* were selected on the basis of maximum *in vivo* expression. These peptides were GFP tagged and selected out from many proteins tested for the *in vivo* expression profile. Vaccination and challenge studies showed that SseB and Mig-14 were exceptionally efficacious antigens providing antigen specific immunity and protection as compared to other antigens used. Other than high expression level, there are certain antigenic parameters which can influence protective efficacy and show different immune response for different antigen[70]. In another DNA vaccine study sopB protein of *Salmonella* was chosen based on its ability to induce better cell-mediated immunity. The vaccine was able to confer protection against *Salmonella* challenge (lethal dose). The protection and immunity was further enhanced when used in combination with live attenuated vaccine candidates[32]. The Table summarizes all the clinical trials which are currently in Phase 1 and II[38,48,53,56,61,71-75].

## Need for new and effective vaccine for typhoid fever

The development of a new vaccine for typhoid fever is an invigorating challenge for the scientists to pursue. Several vaccines have undergone clinical trials but public acceptance is still required. The need of an efficacious and potent vaccine against typhoid fever which can be used in children below 2-5 yr of age providing strong humoral as well as cellular immunity still persists.

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