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Review Article

Typhoid & paratyphoid vaccine development in the laboratory: a review & in-country experience

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Enteric fever is caused by the infection of Gram-negative bacteria, *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi (*S.* Paratyphi) A, B and C, through contaminated food and water. The disease almost exclusively affects the populations living in low- and middle-income countries, with the World Health Organization Southeast Asian Region (WHO SEAR) having the highest endemicity. Despite humans being the sole reservoir of infection and antibiotics and vaccines are made available, the disease was not taken up for elimination until recently due to several biological and technical reasons, including the lack of accurate and region-specific disease surveillance data in the real-time diagnostic inaccuracy of acute infections, difficulty in identifying the chronic asymptomatic carriers who are the major reservoirs of infection and the absence of a political will. However, there is now a renewed interest and effort to control the disease in the endemic areas with the help of better surveillance tools to monitor disease burden, wider availability of more accurate blood culture methods for diagnosis, and above all, cost-effective typhoid conjugate vaccines (TCVs) that can provide a high level of durable protection, particularly against the multidrug-resistant strains and to the age group most commonly affected by the disease. However, despite the commercial availability of a few TCVs, they are still in the development stage. Several questions need to be answered before they are taken up for routine immunization in countries like India. Furthermore, typhoid vaccines with a wider coverage, including additional efficacy against *Salmonella Paratyphi* A and B and preferably the non-typhoidal *Salmonella* (NTS) serovars, for which no vaccines are currently available would be more desirable. We have developed several subunit vaccine candidates containing the glycoconjugates of the surface polysaccharides of typhoidal and non-typhoidal *Salmonellae* and an intrinsic *Salmonella* protein that functions as both antigen and adjuvant. We also developed a novel mouse model of oral *Salmonella* Typhi infection to test the candidate vaccines, which demonstrated broad protective efficacy against *Salmonella spp*. through the induction of humoral and cell-mediated immunity as well as memory response.

Key words Paratyphoid vaccine - *rT2544* - *Salmonella* - typhoid vaccine - vaccine development

Enteric fever, encompassing typhoid and paratyphoid fevers, is a protracted systemic illness caused by Gram-negative pathogens, *Salmonella enterica* serovar Typhi (*S.* Typhi) and *S. enterica* serovar

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Paratyphi A, and rarely by *S*. Paratyphi B and C. They are transmitted through the fecal-oral route and pose significant threats to global public health, especially in the Southeast Asia and Sub-Saharan Africa's low- and middle-income countries (LMICs) with poor standards of potable water supply, sanitation and hygiene (WASH)[1]. This review addresses the need for vaccination against enteric fever in the endemic zones and the journey of the global scientific community, including our own, towards developing affordable and broad-specificity vaccines, capable of providing high level of durable protection.

Disease burden of typhoid and paratyphoid fever: India and the world

The global burden of disease (GBD) study estimated the worldwide incidence and mortality due to typhoid fever in the year 2017 to the tune of 10.9 million and 116.8 thousand, respectively[2]. Corresponding figures for paratyphoid fever during the same time were 3.4 million and 19.1 thousand, respectively[2]. South Asian countries accounted for nearly 70 per cent of all cases and deaths due to enteric fever with children aged 5-9 yr having the highest incidence rates and mortality[2]. However, GBD data may be an underestimate, especially for LMICs[3,4]. Outbreak data, which are frequently uncaptured by community-based studies, could be an additional measure for disease burden estimation[5]. A systematic, global literature search of 303 outbreaks of enteric fever, affecting 1,80,940 individuals between 1990 and 2018 found over 50 per cent of the cases from Asia, but only 46 per cent of the outbreaks reported culture confirmation[5].

Typhoid and paratyphoid fevers continue to be major sources of illness and death in India. GBD (2017) estimated more than 50 per cent of the global typhoid burden from India[2,6]. A systematic review and meta-analysis[7] from India, spanning from 1950 to 2015, documented 377 typhoid and 105 paratyphoid cases per 100,000 person-years, with the highest incidence reported in children of 2-4 yr age group. Other studies reported a similar overall picture but a higher incidence in south-western States and northern urban centers[7]. Interestingly, multiple investigations found the highest incidence in children under five in India, challenging the prevalent notion that typhoid is primarily a disease of older children[8,9]. However, the disease incidence reported in India underscores the significant urban-rural divide, with 576–1173 cases versus 35 cases per 100,000 child-years in the urban versus rural areas[6].

S. Paratyphi A infections comprised only 3–17 per cent of cases in India in the early 1960s[10]. However, recent data indicate a significant increase in the proportion of total enteric fever cases, which exceeded 55 per cent in 2003 and 2004[11]. Similar results were reported in a semi-urban population of West Bengal and from rural Maharashtra[12,13]. A retrospective and a prospective study from Delhi and Chandigarh also confirmed the trend of a significant rise in *S*. Paratyphi A infection. At the same time, the overall number of culture-positive *Salmonella* Typhi remained stable[14,15].

Social and economic cost of enteric fever *versus* the cost of vaccination

Enteric fever is a costly disease for the suffering individuals and their families as well as for the national health systems of the LMICs because of the high disease burden, prolonged disease course and time to complete recovery as well as the cost of antibiotics, especially for multi-drug resistant (MDR) infections[16]. A scoping review[17] of 13 published studies, mainly from Asia between 2000 and 2024 revealed the total cost of a typhoid episode ranging from US$ 23 in India to US$ 884 in Indonesia (as per US$ in 2022), with nine studies characterizing typhoid-related household expenditure as catastrophic. The cost of illness (CoI) also increases substantially for the treatment of severe complications like intestinal perforations (US$ 551 in Niger to US$ 1,735 in India) and drug-resistant infections; USD 223 for extensively drug-resistant (XDR) typhoid in Pakistan[17]. Recently, searches of four databases for studies conducted between 2000-2017 identified 11 CoI, five cost-of-delivery (for the vaccine) and 11 cost-effectiveness analyses (CEA) that compared typhoid treatment and vaccination. Analyses revealed that the costs per outpatient and inpatient cases ranged between US$ 16 and 74 and US$ 159 and 636, respectively, in India[18,19]. However, indirect cost accounted for most of the total CoI, reaching as high as 89 per cent of over US$ 1.3 billion total cost for typhoid fever in LMICs[20]. The high economic burden of typhoid indicates vaccine introduction as a good-value-for-money approach for disease control. For example, the Vi-PS vaccine produced net benefits for mass vaccination or school-based vaccination but was cost-effective (CE) for preschool vaccination in most analyses. However, all Vi-PS vaccination programmes would be very CE if the indirect expenses were also accounted for[20]. Despite limited evidence, Typhoid conjugate vaccine (TCV) was generally found CE for infant routine

immunization programmes in most countries and could prevent new infections and deaths[21].

Typhoid elimination: barriers and opportunities, the vaccine gap

Early and accurate diagnosis of enteric fever remains a challenge to the world, because of the non-specific signs and symptoms. Blood culture is considered the gold standard for diagnosis but of limited usefulness in the clinical setting due to high cost, low yield (40-60% positivity) and prolonged time to get the results. Serological tests (Widal, Typhidot) lack specificity[22], while the newer diagnostic methods, such as PCR or multiplex PCR, ELISA, dot immunoassay, immuno-electrophoresis, haem-agglutination and co-agglutination are promising, but unsuitable for routine clinical use due to technical challenges[22]. Rising antimicrobial resistance, particularly the emergence and spread of multidrug-resistant (MDR - resistant to chloramphenicol, ampicillin, and co-trimoxazole) and extensively drug-resistant (XDR - additional resistance to third-generation cephalosporins) strains posing a major challenge to enteric fever control, especially in the LMICs[23]. It is most alarming that XDR strains replaced all other *Salmonella* Typhi strains in Sindh, Pakistan and has started showing resistance to azithromycin, the sole antibiotic left to deal with them[24]. Although MDR-phenomena is still rare in *S.* Paratyphi, a healthcare facility-based surveillance from Bangladesh reported increased MIC to ciprofloxacin in more than 99 per cent of strains[25]. The high disease burden of enteric fever in the LMICs, accompanied by diagnostic challenges and emerging multidrug resistance, leading to potentially severe complications and lethality, and the disproportionately high social and economic cost of illness, call for heightened activities for disease elimination. While elimination is a long-term goal, reduction of incidence to the locally acceptable level could be achieved within a defined time-period with the improvement of WASH and food safety, availability of point of contact water disinfection techniques, improved surveillance tools for disease burden estimation and efficacy of control measures and blood cultures for diagnosis[26]. However, adoption of the available Ty21a and Vi-PS vaccines in the routine immunization programme of the high endemic countries was poor despite WHO recommendations because of their unsuitability for infants and younger children. These concerns were largely eliminated by the recently commercialized TCVs, which were found to be safe for six-month-old infants and impart higher magnitude and longer duration of protection.

Studies have suggested that TCV introduction into the routine immunization programme in endemic areas at nine months of age with a catch-up campaign to 15 years will be cost-effective, and accounting for the indirect cost of enteric fever would make vaccination even more cost-saving. TCV is not only effective against MDR and XDR strains (95% against culture-confirmed MDR and 97% against XDR *S.* Typhi) but also could reduce antimicrobial resistance of typhoid by ~16 per cent[27]. Several countries, such as Pakistan, Samoa, Liberia, Nepal and Zimbabwe, introduced TCV for routine immunization[28]. Still, its wider acceptance by countries like India would require additional information, including duration of protection and frequency of booster doses, as well as its role in eliminating infection, reducing faecal shedding of *Salmonella* in chronic carriers and providing herd protection.

Vaccine development strategies: A historical perspective

Typhoid vaccines: In 1896, Richard Pfeiffer and Almond Wright independently published their work on the first typhoid fever vaccine – a-heat inactivated whole cell vaccine[29]. This vaccine was successfully and extensively used during World War I[29]. However, local and systemic reactogenicity in the vaccine recipients resulted in its withdrawal from the list of licensed vaccines and routine immunization programmes. After a long gap, live attenuated, oral typhoid vaccine, Ty21a was developed in the late 1980s by chemical mutagenesis of the *S.* Typhi Ty2 strain. Ty21a is modestly immunogenic and requires multiple booster doses for optimal immunogenicity[30]. However, the large capsules make it difficult for children below six years of age to swallow and the need for pre-administration of buffer to neutralize the stomach acid is also a potential delivery challenge[29]. An additional risk of bacteremia was also reported for live-engineered typhoid vaccines. Around the same time, Robins and Robins from NIH, USA developed an injectable Vi-PS vaccine in 1986, followed by WHO prequalification of the vaccine manufactured by Pasteur[31]. Despite an acceptable safety profile, Vi-PS, being a T-independent antigen, is poorly immunogenic, especially for younger children.

Further research was directed towards single-dose typhoid vaccine development by attenuation

of genetically modified *S.* Typhi on one hand and conjugation of Vi-PS to carrier proteins to convert it to a T-dependent antigen on the other. A single oral dose, containing 10^7 viable organisms of CVD 908, an *aroC/aroD* deletion mutant of *S.* Typhi Ty2 was immunogenic, but resulted in vaccinemia. The attenuation was further enhanced by deleting a heat-stress protein, htrA that prevented vaccinemia while retaining both humoral and cellular immune responses[32]. To ensure more consistent serum anti-Vi antibodies, Vi-PS was constitutively expressed in CVD 908 strain, generating CVD 909. However, volunteers receiving one or two oral doses of CVD 909 or a prime boost regimen with oral CVD 909, followed by an injection of Vi-PS vaccine, failed to induce consistent anti-Vi antibody response, although Vi-specific IgA^+ memory B cells were significantly raised[33].

Chemical conjugation of Vi-PS to a carrier protein significantly augmented immunogenicity. Typbar TCV (Vi-TT, Vi-PS conjugated to tetanus toxoid), launched by Bharat Biotech, India, was the first WHO pre-qualified typhoid conjugate vaccine (TCV) and was approved for administration to infants as young as six months of age[34]. Recent TyVac trials with Typbar TCV in Nepal, Bangladesh and Malawi showed protective efficacy of 79 per cent, 85 per cent for upto two years[35,36] and 80 per cent for up to four years[37].

Despite this, the protective antibody titer is still unknown, and in the absence of *Salmonella*-specific cytotoxic T cells generation, clearance of intracellular bacteria remains uncertain[38]. Several other Vi conjugate vaccines, carrying recombinant carrier proteins, such as *Pseudomonas aeruginosa* exotoxin A (rEPA)[39], CRM197[40-43] and diphtheria toxoid[44-46] showed comparable efficacy, but suffer from the same limitations as Vi-TT. Typhoid conjugate vaccines are still under investigations to further improve their efficacy and awaiting approval for wider application (Table[30-36,37,39,41-54] & Fig. 1).

Paratyphoid vaccines: A similar strategy to typhoid vaccines was adopted to develop paratyphoid vaccines. Genetically engineered, live attenuated *S.* Paratyphi A strains were generated by mutating critical target genes, such as *phoP/phoQ, htrA, ssaV and clpPX.* Genetic deletion of *phoP/phoQ* in *S.* Paratyphi A by Roland *et al*[55] in 2010 gave rise to an attenuated strain, which was immunogenic and well tolerated in an oral rabbit model. Another study with SPADD01, containing genetic mutation of *aroC*, critical for amino acid biosynthesis and *yncD*, encoding a TonB-dependent transporter showed significant attenuation, but excellent humoral and mucosal immune response in a mouse model[56]. Researchers introduced an additional mutation of the *htrA* gene in the *yncD* mutant *S.* Paratyphi A, further reducing the virulence[57]. Nasal administration of this double mutant strain protected immunized mice against lethal bacterial challenge[57]. CVD 1902, which incorporated combined mutations in the *guaBA* and *clpX* genes, involved in the de novo synthesis of guanine nucleotides and a chaperon ATPase, respectively, is also an attractive, live attenuated, paratyphoid vaccine candidate. Volunteer trials with single doses of 10^9 or 10^10 CFU of CVD 1902 strain were well tolerated and triggered paratyphi lipopolysaccharide-specific IgG and/or IgA B-memory cells and paratyphi-specific CD8+ and/or CD4+ T effector/memory cells[58].

Besides, subunit vaccine candidates for *S.* Paratyphi, comprising of surface or secretory proteins, such as the outer membrane proteins and O-specific polysaccharide (OSP) exhibited robust immune protection. Systemic immunization with 100 µg to 500 µg of *S.* Paratyphi A outer membrane proteins PagC, LamB, NmpC, TolCFadL and SpaO conferred 60 per cent to 95 per cent protective efficacy against paratyphoid infection, but requires further detailed evaluation of dose optimization and cross-protection against typhoidal infection[59]. Instead of Vi-PS, surface OSP as a protective antigen for *S.* Paratyphi A, which lacks the Vi antigen. OSP conjugates linked to diverse carrier proteins were developed for *S.* Paratyphi A, following the similar strategy employed for Vi conjugate vaccines[48]. In 1996, researchers at the US National Institute of Health (NIH) developed OSP-TT[60], which documented considerable immunogenicity but no significant vaccination-induced side effects in a Vietnamese trial. However, booster response was not observed in children[61]. This technology was transferred to the Lanzhou Institute of Biological Products, and its product has progressed through Phase I and II clinical trials[49]. Other OSP-conjugate vaccines against paratyphoid infection undergoing preclinical evaluation used diphtheria toxoid[62] (International Vaccine Institute, Seoul, Korea) and CRM197, a genetically modified diphtheria toxin (Novartis Vaccine Institute of Global Health, Sienna, Italy) as carrier proteins[63]. Isolation of bacterial OSP requires large-scale fermentation of pathogenic organisms, followed by a detoxification process to eliminate endotoxins. Aiming to bypass the need for detoxification, one research group utilized

Table. Vaccines against typhoid fever

Nature of the vaccine	Name of the vaccine	Modifications in vaccine candidates	Status of the vaccine	Reference
Licensed typhoidal vaccine				
Live attenuated vaccine	Ty21a Vivotif (Crucell)	gal E mutant.	Commercially available	30, 47, 48
Vi based Vaccine	Vi-PS Typherix (GSK), Typhim Vi (Sanofi), Typbar Vi (Bharat Biotech)	Purified Vi polysaccharide.	Commercially available	31, 47
Vi based conjugate Vaccine	Vi-TT, (Typbar TCV, Bharat Biotech, (PedaTyph-Biomed) (ZyVac-TCV, Cadila Healthcare)	Vi polysaccharide is conjugated with the Tetanus toxoid	Prequalified and Recommended by WHO	34, 35, 36, 37
Typhoidal vaccines under development				
Vi based conjugate Vaccine	Vi-DT, PT Biopharma, Vi-DT, SK Bioscience	Vi polysaccharide is conjugated with diphtheria toxoid	Phase III clinical trial. WHO Prequalified on 2024	44 45, 46
	Vi-CRM197 EuBiologicals	Vi polysaccharide is conjugated with the CRM197, a nontoxic mutant of diphtheria toxin.	Phase III clinical trials.	43
	Vi-CRM197 Biological E		WHO Prequalification on 2020	41, 42
	Vi-EPA, Lanzhou Institute of Biological Products	Pseudomonas aeruginosa exotoxin A as a carrier protein.	National Licensure	39, 49, 50
Live attenuated Vaccine	CVD 908 University of Maryland	Deletion in the *aroC* and *aroD*	Showed bacteremia in clinical trials.	51
	CVD 908-htrA University of Maryland	Deletion in the *aroC, aroD* and *htrA* genes	Phase II study with 80 human volunteers.	32
	CVD 909 University of Maryland	Constitutive expression of Vi polysaccharide	Phase I clinical trial	33, 52
	Ty800 Avant Immunotherapeutics	Deletion in the *phoP/ phoQ* virulence regulatory genes.	Phase II clinical trial	53
	M01ZH09 emergent Biosolutions	Deletion in the *aroC* and *ssaV* genes	Phase II clinical trial.	54

WHO, World Health Organization; CVD, University of Maryland Center for Vaccine Development

synthetic oligosaccharides that corresponded to the O-polysaccharide repeating units of *S.* Paratyphi A to construct a glycoconjugate formulation by linking them to a carrier, bacteriophage Qb[64]. This conjugate successfully induced high levels of anti-glycan IgG antibodies in mice, and passive immunization with the antisera protected from lethal challenges with *S.* Paratyphi A[64].

Bivalent vaccines: Since endemic areas of *Salmonella* Typhi and Paratyphi infections largely overlap, bivalent vaccine candidates targeting both organisms are in high demand[65]. Mass immunization with Vi conjugate vaccines may exert selection pressure on the existing Vi-negative strains, eventually making vaccination ineffective[38]. A prospective study[65] in Guangxi, China, found a significant shift from *S.* Typhi to *S.* Paratyphi A outbreaks three years after introducing Vi-based vaccines. Data on the efficacy of the oral Ty21a vaccine against paratyphoid infections are inconsistent[66-67]. OSP O2-conjugates of *S.* Paratyphi A were combined with Vi-TT, Vi-CRM197 or Vi-DT for wider protection[68]. An exciting alternative to the traditional conjugation methods is the Multiple Antigen Presenting System (MAPS), which uses the biotin-rhizavidin affinity pair to create a complex of polysaccharides and proteins. Vaccines based on MAPS generate functional antibodies and Th1/Th17

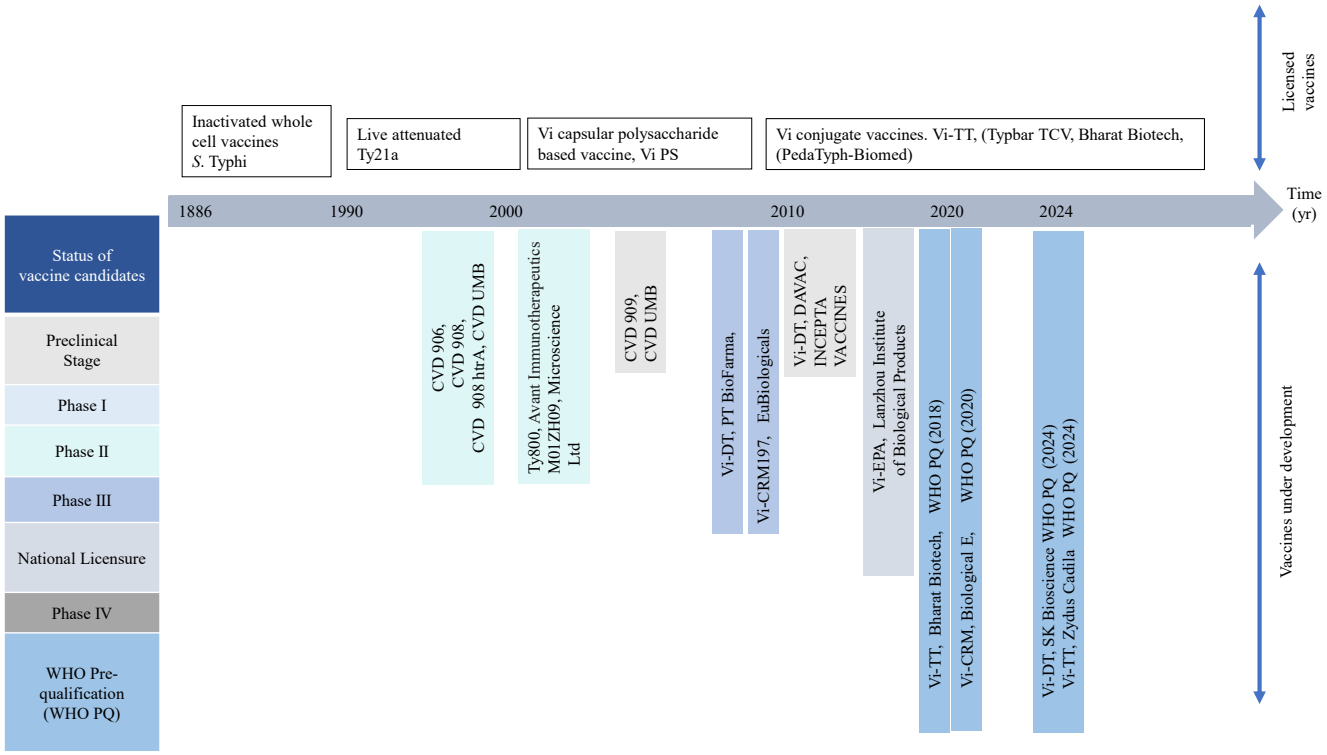

Fig. 1. Typhoid vaccine pipeline. This figure provides an overview of the current status and development stages of typhoid vaccine candidates. CVD-UMB, Center for vaccine development University of Maryland; ViPS, Vi capsular polysaccharide; Vi-EPA, Vi polysaccharide conjugated to *Pseudomonas aeruginosa* exotoxin A (rEPA); Vi-DT, Vi polysaccharide conjugated to diphtheria toxoid.

cell responses. A bivalent vaccine targeting Vi and OSP was developed using the MAPS that contained a fusion of three proteins, CRM197, Pseudomonas rEPA, and pneumococcal SP1500-SP0785 to Rhizavidin. This vaccine demonstrated significantly higher affinity maturation of both Vi and OSP antibodies with minimal cross-interference functionally when compared with the monovalent vaccine[69]. Recent investigations used an engineered *S.* Paratyphi A, utilizing pDC5-viaB plasmid to produce GMMA that displayed *S.* Typhi Vi antigen and the O:2 antigen from Paratyphi A and elicited strong humoral responses and bactericidal activity against both pathogens, supporting its potential use for enteric fever control[70,71,72] (Supplementary Table I).

Our vaccine development efforts as a case study: Near the end of the first decade of the new millennium when we started our journey for *Salmonella* vaccine development, there were only two licensed *Salmonella* vaccines – live, attenuated Ty21a and injectable Vi-polysaccharide (Vi-PS) vaccines, meant for use against only *Salmonella* Typhi, although not suitable for young children. Both vaccines offered inconsistent cross-protection against *Salmonella* Paratyphi A and B[73-75]. This prompted us to consider protein subunit-based vaccine development that could simultaneously protect against *Salmonella* Typhi and Paratyphi infections. Through advanced bioinformatics and experimental techniques, our team identified several promising candidates, finally leading to the discovery of a significant protein, called T2544[76]. Computational prediction of the three-dimensional structure of this 27-kDa outer membrane protein revealed membrane embedded β-sheets and externally projected α-helices that bind to the host extracellular matrix protein, laminin. However, that T2544-laminin binding was essential for bacterial virulence and T2544-based subunit vaccine could protect against intestinal *Salmonella* infection required an animal model that was not available at that moment for the human restricted enteric fever pathogens, except for primates[76]. Literature searches gave us the impression that the *in vivo* availability of elemental iron might be the limiting factor for typhoidal *Salmonellae* to establish rodent infection[77]. Previous research had shown that host siderophore, NRAMP-1 mutant mouse, was exquisitely susceptible to *Salmonella* infection[78], while systemic iron overload increased the susceptibility of wild-type mouse strain to *S.* Typhi infection[77,79]. However, the use of a large

dose of iron often results in immunosuppression and lethality due to organ toxicities[80], which could be avoided by the co-administration of iron and iron chelator, desferrioxamine that makes the element iron (Fe^{3+}) available to the intracellular bacteria, promoting their survival and growth[79]. We standardized a paired dose of iron (0.32 mg per gm of body weight) and Desferal (25 mg/Kg body weight) that limited iron toxicity but established infection in wild-type BALB/c mouse after oral gavage with *S.* Typhi. Similar to humans, liver, spleen and the bone marrow were the primary visceral organs affected in the mouse, suggesting that this might be considered a physiological model for *Salmonella* Typhi infection. The model developed fulfilled a long-standing demand for a rodent model of typhoid after infection through the natural route, which could serve the dual purpose of studying intestinal pathogenesis and immune response. Immunization of mice with the candidate subunit vaccine indeed induced raised intestinal secretory IgA levels that decreased gut colonization by *S.* Typhi[81]. While the induction of intestinal immune response following systemic vaccine administration was reported earlier[76], its protective role *in vivo* was not demonstrated. In addition, immunized mice developed high titers of T2544-specific opsonic antisera, which augmented complement-mediated lysis, phagocytosis by the macrophages and antibody-dependent cellular cytotoxicity (ADCC) of the bacteria[82] and conferred protection after passive immunization. Most impressively, acute and convalescent typhoid patients' sera containing significantly raised titers of T2544-specific bactericidal antibodies could be neutralized by adsorption with T2544, suggesting that it is an immunodominant antigen for the human infection[76]. Our subsequent studies revealed that the candidate vaccine could also elicit T2544-specific cell-mediated immune response, including the T helper 1 (Th1) cells and cytotoxic T lymphocytes (CTLs)[82]. Together, our research findings underscored the importance of T2544 in orchestrating an effective immune response to human pathogenic *Salmonella spp.* This was further supported by significant protection of mice immunized with recombinant T2544-based candidate vaccine or passively administered with T2544 antiserum against *S.* Typhi[82]. In addition to considering the apparent advantage of a protein subunit vaccine compared with the polysaccharide-based formulations for younger children and the presumed protection conferred by T2544 against *S.* Paratyphi A infection, this candidate vaccine was patented by us (Patent no. 283894; dated 09.09.2011) to ensure retention of its intellectual property within India.

However, we failed to identify an interested industrial partner for further development of the candidate vaccine to commercialize it. This was perhaps influenced by intense research to develop Vi-polysaccharide-based typhoid conjugate vaccines (TCVs) during that period. The success of capsular polysaccharide-based conjugate vaccines against Hib, pneumococci and meningococci fuelled this interest. TCVs demonstrated excellent safety profile and robust and durable antibacterial immunity in children as young as 6-9 months of age[37,83]. Typhi Vi polysaccharide conjugated to tetanus toxoid from Bharat Biotech in December 2017, followed by TYPHIBHEV (Vi polysaccharide from *Citrobacter freundii* conjugated to CRM) by Biological E in December 2020 and SKY Typhoid (*S.* Typhi Vi polysaccharide conjugated to diphtheria toxoid) in February 2024 that was marketed by SK Biosciences. However, concerns were raised against the carrier proteins most commonly used for TCVs, namely the tetanus toxoid and diphtheria toxoid, also used as vaccine antigens in the routine immunization programme for children or as carrier proteins for several conjugate vaccines. Simultaneous or sequential use of the same carrier protein as a part of multiple conjugate vaccines or as a vaccine antigen and part of a conjugate vaccine may lead to decreased immunogenicity of the co-administered antigen due to antigenic competition or carrier-induced epitope suppression (CIES)[84,85]. For example, vaccination with PCV13 and MCV4, 3-4 wk after Tdap vaccine significantly reduced the geometric mean titer to seven of the 13 pneumococcal serotypes in adults[86] and priming with DT suppressed the response to DT-MenA conjugates[87]. Several mechanisms have been implicated for this immune interference, including carrier specific B cells expansion during priming, followed by competition with the co-administered antigen-specific B cells, presentation of the carrier-polysaccharide conjugate by the B-cells as opposed to dendritic cells after pre-immunization, competition for antigen and antigen-bearing cells and the development of carrier-specific suppressor T cells during priming that can induce suppressor T cells specific for the conjugated antigen after immunization[88]. To overcome such problems, we replaced TT/DT with recombinant T2544 as the provider of the T cell helper epitopes for the new TCV. Given that T2544 is a protective antigen, this approach would add an 'additional valency', which is generally neglected for conjugate vaccines and

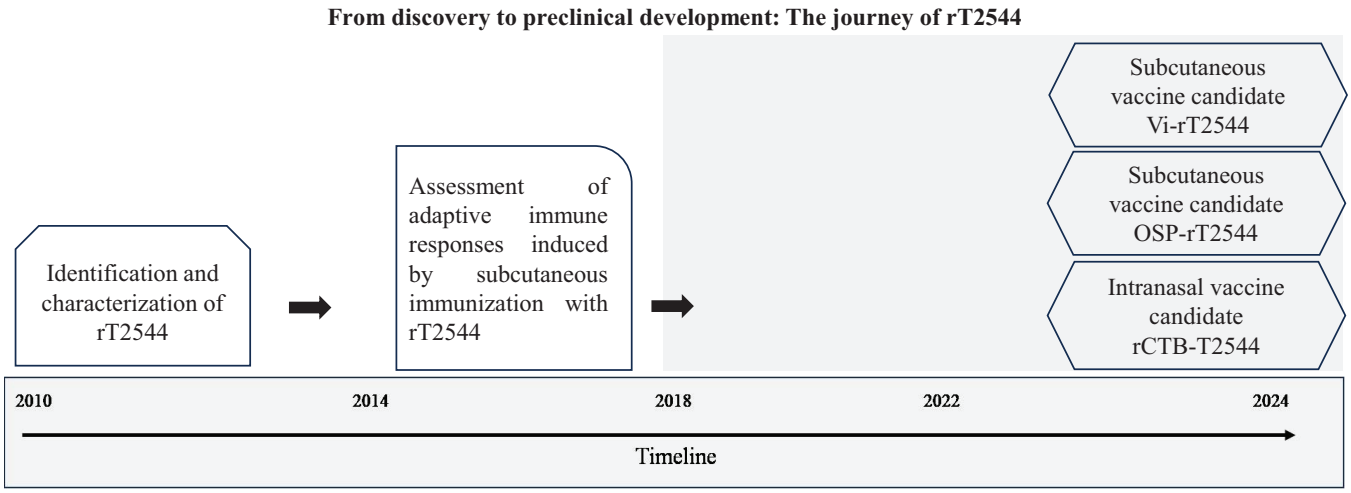

Fig. 2. Progression of vaccine candidate from discovery to preclinical development. This figure maps the path of our vaccine candidates, tracing its evolution from initial discovery to preclinical development stage. rT2544, outer membrane protein of *Salmonella* Typhi and Paratyphi; rCTB-T2544, Cholera toxin B genetically fused to T2544; OSP-rT2544, O-polysaccharide of *S.* Typhimurium chemically linked to T2544; Vi-rT2544, Vi polysaccharide chemically linked to T2544.

further augment the immune response. To check for the immune adjuvant functions of T2544, we immunized mice with Vi-PS along with recombinant T2544. This led to modest increase in Vi-PS specific serum IgG titers. Several studies had indicated that most adjuvants work better when covalently conjugated to the antigens rather than co-administered as a mixture[38,89]. However, solubility of T2544 was challenge which we finally succeeded in overcoming. Serum SBA titer was greater for Vi-T2544, which conferred better protection to mice against *S.* Typhi infection than Vi-TT with a wider coverage that includes paratyphoid infection (Fig. 2 & Supplementary Table II). A patent application for the candidate vaccine formulation containing Vi-T2544 has been filed to the Indian Patent Office (IPO) (Application number 202411074276; filing date: October 1, 2024).

To further extend vaccine-induced protection to the non-typhoidal *Salmonella* (NTS) serovars, we considered conjugating recombinant T2544 protein to *S.* Typhimurium O-specific polysaccharide (OSP)[90]. O-specific polysaccharides (OSPs) from different pathogens, conjugated to different carrier proteins (such as TT, DT, CRM197, and FliC) have demonstrated protective efficacy, while unconjugated OSP exhibits limited immunogenicity[62,63,91,92]. Similar to Vi-PS, T2544 displayed strong adjuvant function to OSP and subcutaneous immunization of mice with OSP-T2544 candidate vaccine conferred protection against *Salmonella* Typhi, Paratyphi and Typhimurium. However, more intriguing was the cross-protection against *Salmonella* enteritidis[90], because the side chains attached to the common backbone of O-antigens from different serovars that confer distinct antigenic specificity are different for *S. Typhimurium* and *S. Eneteritidis*[93,94]. While the mechanisms behind cross-reactivity to *S. enteritidis* remain under investigation, it is possible that antibodies directed against the conserved O-antigen epitopes, such as O:1 and O:12, or the shared core region, contribute to it. We were also impressed by the strong recall response after the vaccination, characterized by higher titers and avidity of serum IgG against both OSP and T2544 that ensured long-term protection. Protection was also correlated with the serum bactericidal antibodies (SBA) titers and bacterial motility inhibition by intestinal secretory IgA. An Indian patent application is currently pending (Patent application number 202311070211; filing date: October 16, 2023).

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References

1. Hancuh M, Walldorf J, Minta AA, Tevi-Benissan C, Christian KA, Nedelec Y, *et al*. Typhoid fever surveillance, incidence estimates, and progress toward typhoid conjugate vaccine introduction - Worldwide, 2018-2022. *MMWR Morb Mortal Wkly Rep* 2023; *72* : 171-176.
2. Typhoid GBD, Paratyphoid C. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the global burden of disease study 2017. *Lancet Infect Dis* 2019; *19* : 369-81.
3. Antillón M, Warren JL, Crawford FW, Weinberger DM, Kürüm E, Pak GD, *et al*. The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. *PLoS Negl Trop Dis* 2017; *11* : e0005376.
4. Marchello CS, Hong CY, Crump JA. Global typhoid fever incidence: A systematic review and meta-analysis. *Clin Infect Dis* 2019; *68* : S105-16.
5. Kim S, Lee KS, Pak GD, Excler JL, Sahastrabuddhe S, Marks F, *et al*. Spatial and temporal patterns of typhoid and paratyphoid fever outbreaks: A worldwide review, 1990-2018. *Clin Infect Dis* 2019; *69* : S499-S509.
6. John J, Bavdekar A, Rongsen-Chandola T, Dutta S, Gupta M, Kanungo S, *et al*. Burden of typhoid and paratyphoid fever in India. *N Engl J Med* 2023; *388* :1491-1500.
7. Cao Y, Karthikeyan AS, Ramanujam K, Raju R, Krishna S, Kumar D, *et al*. Geographic pattern of typhoid fever in India: A model-based estimate of cohort and surveillance data. *J Infect Dis* 2021; *224* : S475-83.
8. John J, Van Aart CJ, Grassly NC. The burden of typhoid and paratyphoid in India: Systematic review and meta-analysis. *PLoS Negl Trop Dis* 2016; *10* : e0004616.
9. Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, *et al*. Typhoid fever in children aged less than 5 years. *Lancet* 1999; *354* :734-7.
10. Saxena SN, Sen R. Salmonella paratyphi A infection in India: Incidence and phage types. *Trans R Soc Trop Med Hyg* 1966; *60* : 409-11.
11. Verma S, Thakur S, Kanga A, Singh G, Gupta P. Emerging salmonella paratyphi A enteric fever and changing trends in antimicrobial resistance pattern of Salmonella in Shimla. *Indian J Med Microbiol* 2010; *28* :51-3.
12. Palit A, Ghosh S, Dutta S, Sur D, Bhattacharya MK, Bhattacharya SK. Increasing prevalence of Salmonella enterica serotype Paratyphi-A in patients with enteric fever in a periurban slum setting of Kolkata, India. *Int J Environ Health Res* 2006; *16* : 455-9.
13. Mendiratta DK, Deotale V, Thamke D, Narang R, Narang P. Enteric fever due to S. paratyphia - an emerging problem. *Indian J Med Microbiol* 2004; *22* :196.
14. Mohanty S, Renuka K, Sood S, DAS BK, Kapil A. Antibiogram pattern and seasonality of Salmonella serotypes in a North Indian tertiary care hospital. *Epidemiol Infect* 2006; *134* : 961-6.
15. Gupta V, Kaur J, Chander J. An increase in enteric fever cases due to Salmonella Paratyphi A in & around Chandigarh. *Indian J Med Res* 2009; *129* : 95-8.
16. Kuehn R, Stoesser N, Eyre D, Darton TC, Basnyat B, Parry CM. Treatment of enteric fever (typhoid and paratyphoid fever) with cephalosporins. *Cochrane Database Syst Rev* 2022; *11* : CD010452.
17. Debellut F, Friedrich A, Baral R, Pecenka C, Mugisha E, Neuzil KM. The cost of typhoid illness in low- and middle-income countries, a scoping review of the literature. *PLoS One* 2024; *19* : e0305692.
18. Poulos C, Riewpaiboon A, Stewart JF, Clemens J, Guh S, Agtini M, *et al*. Cost of illness due to typhoid fever in five Asian countries. *Trop Med Int Health* 2011; *16* : 314-23.
19. Bahl R, Sinha A, Poulos C, Whittington D, Sazawal S, Kumar R, *et al*. Costs of illness due to typhoid fever in an Indian urban slum community: Implications for vaccination policy. *J Health Popul Nutr* 2004; *22* :304-10.
20. Luthra K, Watts E, Debellut F, Pecenka C, Bar-Zeev N, Constenla D. A Review of the economic evidence of typhoid fever and typhoid vaccines. *Clin Infect Dis* 2019; *68* : S83-S95.
21. Chauhan AS, Kapoor I, Rana SK, Kumar D, Gupta M, John J, *et al*. Cost effectiveness of typhoid vaccination in India. *Vaccine* 2021; *39* : 4089-98.
22. Mukhopadhyay B, Sur D, Gupta SS, Ganguly NK. Typhoid fever: Control & challenges in India. *Indian J Med Res* 2019; *150* : 437-47.
23. Qamar FN, Yousafzai MT, Dehraj IF, Shakoor S, Irfan S, Hotwani A, *et al*. Antimicrobial resistance in typhoidal Salmonella: Surveillance for enteric fever in Asia project, 2016-2019. *Clin Infect Dis* 2020; *71* : S276-S284.
24. Butt MH, Saleem A, Javed SO, Ullah I, Rehman MU, Islam N, *et al*. Rising XDR-Typhoid fever cases in Pakistan: Are we heading back to the pre-antibiotic era? *Front Public Health* 2022; *9* : 794868.
25. Sajib MSI, Tanmoy AM, Hooda Y, Rahman H, Munira SJ, Sarkar A, *et al*. Trends in antimicrobial resistance amongst Salmonella Paratyphi A isolates in Bangladesh: 1999-2021. *PLoS Negl Trop Dis* 2023; *17* : e0011723.
26. Stanaway JD, Atuhebwe PL, Luby SP, Crump JA. Assessing the feasibility of typhoid elimination. *Clin Infect Dis* 2020; *71* : S179-84.
27. Yousafzai MT, Karim S, Qureshi S, Kazi M, Memon H, Junejo A, *et al*. Effectiveness of typhoid conjugate vaccine against culture-confirmed Salmonella enterica serotype Typhi in an extensively drug-resistant outbreak setting of Hyderabad, Pakistan: A cohort study. *Lancet Glob Health* 2021; *9* : e1154-62.
28. Chen J, Long JE, Vannice K, Shewchuk T, Kumar S, Duncan Steele A, *et al*. Taking on Typhoid: Eliminating typhoid fever as a global health problem. *Open Forum Infect Dis* 2023; *10* : S74-S81.

29. Williamson JD, Gould KG, Brown K. Richard Pfeiffer's typhoid vaccine and Almroth Wright's claim to priority. *Vaccine* 2021; *39* : 2074-9.
30. Syed KA, Saluja T, Cho H, Hsiao A, Shaikh H, Wartel TA, *et al*. Review on the recent advances on typhoid vaccine development and challenges ahead. *Clin Infect Dis* 2020; *71* : S141-S150.
31. Date KA, Bentsi-Enchill A, Marks F, Fox K. Typhoid fever vaccination strategies. *Vaccine* 2015; *33* : C55-61.
32. Tacket CO, Sztein MB, Wasserman SS, Losonsky G, Kotloff KL, Wyant TL, *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; *68* : 1196-201.
33. Wahid R, Pasetti MF, Maciel M, Simon JK, Tacket CO, Levine MM, *et al*. Oral priming with Salmonella Typhi vaccine strain CVD 909 followed by parenteral boost with the S. Typhi Vi capsular polysaccharide vaccine induces CD27+IgD-S. Typhi-specific IgA and IgG B memory cells in humans. *Clin Immunol* 2011; *138* : 187-200.
34. World Health Organization. Typhoid vaccines: WHO position paper, March 2018 - Recommendations. *Vaccine* 2019; *37* : 214-16.
35. Shakya M, Voysey M, Theiss-Nyland K, Colin-Jones R, Pant D, Adhikari A, *et al*. Efficacy of typhoid conjugate vaccine in Nepal: Final results of a phase 3, randomized, controlled trial. *Lancet Glob Health* 2021; *9* : e1561-8.
36. Khanam F, Kim DR, Liu X, Voysey M, Pitzer VE, Zaman K, *et al*. Assessment of vaccine herd protection in a cluster-randomized trial of Vi conjugate vaccine against typhoid fever: Results of further analysis. *EClinicalMedicine* 2023; *58* : 101925.
37. Patel PD, Liang Y, Meiring JE, Chasweka N, Patel P, Misiri T, *et al*. Efficacy of typhoid conjugate vaccine: Final analysis of a 4-year, phase 3, randomized controlled trial in Malawian children. *Lancet* 2024; *403 :* 459-68.
38. Simon R, Levine MM. Glycoconjugate vaccine strategies for protection against invasive Salmonella infections. *Hum Vaccin Immunother* 2012; *8* : 494-498.
39. Thiem VD, Lin FY, Canh DG, Son NH, Anh DD, Mao ND, *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 :* 730-5.
40. Bhutta ZA, Capeding MR, Bavdekar A, Marchetti E, Ariff S, Soofi SB, *et al*. Immunogenicity and safety of the Vi-CRM197 conjugate vaccine against typhoid fever in adults, children, and infants in south and southeast Asia: Results from two randomized, observer-blind, age de-escalation, phase 2 trials. *Lancet Infect Dis* 2014; *14* : 119-29.
41. Shakya M, Pollard AJ. Typhoid conjugate vaccines: a step towards typhoid control. *Lancet Glob Health* 2024; *12* : e535-6.
42. ThuluvaS, Paradkar V, Matur R, Turaga K, Gv SR. A multicenter, single-blind, randomized, phase-2/3 study to evaluate immunogenicity and safety of a single intramuscular dose of biological E's Vi-capsular polysaccharide-CRM(197) conjugate typhoid vaccine (TyphiBEV(TM)) in healthy infants, children, and adults in comparison with a licensed comparator. *Hum Vaccin Immunother* 2022; *18* : 2043103.
43. Ok Baik Y, Lee Y, Lee C, Kyung Kim S, Park J, Sun M, *et al*. A phase ii/iii, multicenter, observer-blinded, randomized, non-inferiority and safety, study of typhoid conjugate vaccine (EuTCV) compared to Typbar-TCV® in healthy 6 months-45 years aged participants. *Vaccine* 2023; *41 :* 1753-59.
44. Medise BE, Soedjatmiko S, Rengganis I, Gunardi H, Sekartini R, Koesno S, *et al*. Six-month follow up of a randomized clinical trial-phase I study in Indonesian adults and children: Safety and immunogenicity of Salmonella typhi polysaccharide-diphtheria toxoid (Vi-DT) conjugate vaccine. *PLoS One* 2019; *14* : e0211784.
45. Kumar Rai G, Saluja T, Chaudhary S, Tamrakar D, Kanodia P, Giri BR, *et al*. Safety and immunogenicity of the Vi-DT typhoid conjugate vaccine in healthy volunteers in Nepal: An observer-blind, active-controlled, randomized, non-inferiority, phase 3 trial. *Lancet Infect Dis* 2022; *22* : 529-40.
46. Capeding MR, Tadesse BT, Sil A, Alberto E, Kim DR, Park EL, *et al*. Immune persistence and response to booster dose of Vi-DT vaccine at 27.5 months post-first dose. *NPJ Vaccines* 2022; *7* : 12.
47. Milligan R, Paul M, Richardson M, Neuberger A. Vaccines for preventing typhoid fever. *Cochrane Database Syst Rev* 2018; *5* : CD001261.
48. Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, Cryz S. Duration of efficacy of Ty21a, attenuated Salmonella typhi live oral vaccine. *Vaccine* 1999; *17* : S22-7.
49. Shakya M, Neuzil KM, Pollard AJ. Prospects of future typhoid and paratyphoid vaccines in endemic countries. *J Infect Dis* 2021; *224* : S770-4.
50. MacLennan CA, Stanaway J, Grow S, Vannice K, Steele AD. Salmonella combination vaccines: Moving beyond typhoid. *Open Forum Infect Dis* 2023; *10* : S58-S66.
51. Tacket CO, Hone DM, Losonsky GA, Guers L, Edelman R, Levine MM. Clinical acceptability and immunogenicity of CVD 908 *Salmonella typhi* vaccine strain. *Vaccine* 1992; *10* : 443-6.
52. 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* : 565-70.
53. Hohmann EL, Oletta CA, Killeen KP, Miller SI. phoP/phoQ-deleted Salmonella typhi (Ty800) is a safe and immunogenic single-dose typhoid fever vaccine in volunteers. *J Infect Dis* 1996; *173* : 1408-14.
54. Tran TH, Nguyen TD, Nguyen TT, Ninh TT, Tran NB, Nguyen VM, *et al*. A randomized trial evaluating the safety and immunogenicity of the novel single oral dose typhoid vaccine M01ZH09 in healthy Vietnamese children. *PLoS One* 2010; *5* : e11778.
55. Roland KL, Tinge SA, Kochi SK, Thomas LJ, Killeen KP. Reactogenicity and immunogenicity of live attenuated

Salmonella enterica serovar Paratyphi A enteric fever vaccine candidates. *Vaccine* 2010; *28* : 3679-87.

56. Xiong K, Chen Z, Zhu C, Li J, Hu X, Rao X, *et al*. Safety and immunogenicity of an attenuated *Salmonella enterica* serovar Paratyphi A vaccine candidate. *Int J Med Microbiol* 2015; *305* : 563-71.
57. Zhu C, Xiong K, Chen Z, Hu X, Li J, Wang Y, *et al*. Construction of an attenuated *Salmonella enterica* serovar Paratyphi A vaccine strain harboring defined mutations in htrA and yncD. *Microbiol Immunol* 2015; *59* : 443-51.
58. Wahid R, Kotloff KL, Levine MM, Sztein MB. Cell mediated immune responses elicited in volunteers following immunization with candidate live oral *Salmonella enterica* serovar Paratyphi A attenuated vaccine strain CVD 1902. *Clin Immunol* 2019; *201* : 61-9.
59. Yang TC, Ma XC, Liu F, Lin LR, Liu LL, Liu GL, *et al*. Screening of the Salmonella paratyphi A CMCC 50973 strain outer membrane proteins for the identification of potential vaccine targets. *Mol Med Rep* 2012; *5* : 78-83.
60. Konadu E, Shiloach J, Bryla DA, Robbins JB, Szu SC. Synthesis, characterization, and immunological properties in mice of conjugates composed of detoxified lipopolysaccharide of Salmonella paratyphi A bound to tetanus toxoid with emphasis on the role of O acetyls. *Infect Immun* 1996; *64* : 2709-15.
61. Konadu EY, Lin FY, Hó VA, Thuy NT, Van Bay P, Thanh TC, *et al*. Phase 1 and phase 2 studies of *Salmonella enterica* serovar paratyphi A O-specific polysaccharide-tetanus toxoid conjugates in adults, teenagers, and 2- to 4-year-old children in Vietnam. *Infect Immun* 2000; *68* : 1529-34.
62. Ali A, An SJ, Cui C, Haque A, Carbis R. Synthesis and immunogenicity evaluation of *Salmonella enterica* serovar Paratyphi A O-specific polysaccharide conjugated to diphtheria toxoid. *Hum Vaccin Immunother* 2014; *10* : 1494-8.
63. Micoli F, Rondini S, Gavini M, Lanzilao L, Medaglini D, Saul A, *et al*. O:2-CRM(197) conjugates against Salmonella Paratyphi A. *PLoS One* 2012; *7* : e47039.
64. Dhara D, Baliban SM, Huo CX, Rashidijahanabad Z, Sears KT, Nick ST, *et al*. Syntheses of Salmonella Paratyphi A associated oligosaccharide antigens and development towards anti-paratyphoid fever vaccines. *Chemistry* 2020; *26* : 15953-68.
65. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid Fever. *Clin Infect Dis* 2010; *50* : 241-6.
66. Levine MM, Ferreccio C, Black RE, Lagos R, San Martin O, Blackwelder WC. Ty21a live oral typhoid vaccine and prevention of paratyphoid fever caused by Salmonella enterica Serovar Paratyphi B. *Clin Infect Dis* 2007; *45* : S24-8.
67. Gibani MM, Jin C, Shrestha S, Moore M, Norman L, Voysey M, *et al*. Homologous and heterologous re-challenge with Salmonella Typhi and Salmonella Paratyphi A in a randomized controlled human infection model. *PLoS Negl Trop Dis* 2020; *14* : e0008783.
68. Martin LB, Simon R, MacLennan CA, Tennant SM, Sahastrabuddhe S, Khan MI. Status of paratyphoid fever vaccine research and development. *Vaccine* 2016; *34* : 2900-2.
69. Zhang F, Boerth EM, Gong J, Ma N, Lucas K, Ledue O, *et al*. A Bivalent MAPS vaccine induces protective antibody responses against Salmonella Typhi and Paratyphi A. *Vaccines (Basel)* 2022; *11* : 91.
70. Meloni E, Colucci AM, Micoli F, Sollai L, Gavini M, Saul A, *et al*. Simplified low-cost production of O-antigen from Salmonella Typhimurium Generalized Modules for Membrane Antigens (GMMA). *J Biotechnol* 2015; *198* : 46-52.
71. Gasperini G, Alfini R, Arato V, Mancini F, Aruta MG, Kanvatirth P, *et al*. Salmonella paratyphia outer membrane vesicles displaying vi polysaccharide as a multivalent vaccine against enteric fever. *Infect Immun* 2021; *89* : e00699-20.
72. Martin LB, Khanam F, Qadri F, Khalil I, Sikorski MJ, Baker S. Vaccine value profile for *Salmonella enterica* serovar Paratyphi A. *Vaccine* 2023; *41* : S114-33.
73. Wahid R, Simon R, Zafar SJ, Levine MM, Sztein MB. Live oral typhoid vaccine Ty21a induces cross-reactive humoral immune responses against *Salmonella enterica* serovar Paratyphi A and S. Paratyphi B in humans. *Clin Vaccine Immunol* 2012; *19* : 825-34.
74. Pakkanen SH, Kantele JM, Kantele A. Cross-reactive immune response induced by the Vi capsular polysaccharide typhoid vaccine against *Salmonella* Paratyphi strains. *Scand J Immunol* 2014; *79* : 222-9.
75. Zuckerman JN, Hatz C, Kantele A. Review of current typhoid fever vaccines, cross-protection against paratyphoid fever, and the European guidelines. *Expert Rev Vaccines* 2017; *16* : 1029-43.
76. Ghosh S, Chakraborty K, Nagaraja T, Basak S, Koley H, Dutta S, *et al*. An adhesion protein of Salmonella enterica serovar Typhi is required for pathogenesis and potential target for vaccine development. *Proc Natl Acad Sci USA* 2011; *108* : 3348-53.
77. O'Brien AD. Innate resistance of mice to *Salmonella typhi* infection. *Infect Immun* 1982; *38* : 948-52.
78. Fritsche G, Nairz M, Libby SJ, Fang FC, Weiss G. Slc11a1 (Nramp1) impairs growth of Salmonella enterica serovar typhimurium in macrophages *via* stimulation of lipocalin-2 expression. *J Leukoc Biol* 2012; *92* : 353-9.
79. Jones RL, Peterson CM, Grady RW, Kumbaraci T, Cerami A, Graziano JH. Effects of iron chelators and iron overload on Salmonella infection. *Nature* 1977; *267* : 63-5.
80. Eid R, Arab NT, Greenwood MT. Iron mediated toxicity and programmed cell death: A review and a re-examination of existing paradigms. *Biochim Biophys Acta Mol Cell Res* 2017; *1864* : 399-430.
81. Chakraborty S, Dutta P, Pal A, Chakraborty S, Banik G, Halder P, *et al*. Intranasal immunization of mice with chimera of Salmonella Typhi protein elicits protective intestinal immunity. *NPJ Vaccines* 2024; *9* : 24.

82. Das S, Chowdhury R, Ghosh S, Das S. A recombinant protein of Salmonella Typhi induces humoral and cell-mediated immune responses including memory responses. *Vaccine* 2017; *35* : 4523-31.

83. Capeding MR, Sil A, Tadesse BT, Saluja T, Teshome S, Alberto E, *et al*. Safety and immunogenicity of Vi-DT conjugate vaccine among 6-23-month-old children: Phase II, randomized, dose-scheduling, observer-blind study. *EClinicalMedicine* 2020; *27* : 100540.

84. Buttery JP, Riddell A, McVernon J, Chantler T, Lane L, Bowen-Morris J, *et al*. Immunogenicity and safety of a combination pneumococcal-meningococcal vaccine in infants: A randomized controlled trial. *JAMA* 2005; *293* : 1751-8.

85. Tejedor JC, Moro M, Ruiz-Contreras J, Castro J, Gómez-Campderá JA, Navarro ML, *et al*. Immunogenicity and reactogenicity of primary immunization with a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Haemophilus influenzae type B vaccine co-administered with two doses of a meningococcal C-tetanus toxoid conjugate vaccine. *Pediatr Infect Dis J* 2006; *25* : 713-20.

86. Tashani M, Heron L, Wong M, Rashid H, Booy R. Tetanus-diphtheria-pertussis vaccine may suppress the immune response to subsequent immunization with pneumococcal CRM197-conjugate vaccine (co-administered with quadrivalent meningococcal TT-conjugate vaccine): a randomized, controlled trial. *J Travel Med* 2017; 24.

87. Pecetta S, Lo Surdo P, Tontini M, Proietti D, Zambonelli C, Bottomley MJ, *et al*. Carrier priming with CRM 197 or diphtheria toxoid has a different impact on the immunogenicity of the respective glycoconjugates: Biophysical and immunochemical interpretation. *Vaccine* 2015; *33* : 314-20.

88. Jegerlehner A, Wiesel M, Dietmeier K, Zabel F, Gatto D, Saudan P, *et al*. Carrier induced epitopic suppression of antibody responses induced by virus-like particles is a dynamic phenomenon caused by carrier-specific antibodies. *Vaccine* 2010; *28* : 5503-12.

89. Baldauf KJ, Royal JM, Hamorsky KT, Matoba N. Cholera toxin B: one subunit with many pharmaceutical applications. *Toxins (Basel)* 2015; *7* : 974-96.

90. Haldar R, Dhar A, Ganguli D, Chakraborty S, Pal A, Banik G, *et al.* A candidate glycoconjugate vaccine induces protective antibodies in the serum and intestinal secretions, antibody recall response and memory T cells and protects against both typhoidal and non-typhoidal Salmonella serovars. *Front Immunol* 2023; *14* : 1304170 .

91. Baliban SM, Curtis B, Toema D, Tennant SM, Levine MM, Pasetti MF, *et al.* Immunogenicity and efficacy following sequential parenterally-administered doses of Salmonella Enteritidis COPS:FliC glycoconjugates in infant and adult mice. *PLoS Negl Trop* Dis 2018; *12* : e0006522.

92. Ravenscroft N, Cescutti P, Gavini M, Stefanetti G, MacLennan CA, Martin LB, Micoli F. Structural analysis of the O-acetylated O-polysaccharide isolated from Salmonella paratyphi A and used for vaccine preparation. *Carbohydr Res* 2015; *404* : 108-16.

93. Fiorino F, Rondini S, Micoli F, Lanzilao L, Alfini R, Mancini F, *et al*. Immunogenicity of a bivalent adjuvanted glycoconjugate vaccine against *Salmonella* typhimurium and *Salmonella* enteritidis. *Front Immunol* 2017; *8* : 168.

94. World Health Organization. *WHO collaborating centre for reference and research on Salmonella. Antigenic formulae of the Salmonella serovars (2007).* Available from: *https://www.pasteur.fr/sites/default/files/veng_0.pdf*, accessed on November 6, 2022.

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Supplementary Table I. Bivalent vaccines

Vaccine candidates	Developer	Modification	Route of delivery	Current Status	Reference
CVD 1902 with CVD 909	University of Maryland Center for Vaccine Development and Global Health (CVD) And Bharat Biotech International Limited	CVD 1902: Deletions in the guaBA and clpX regions. CVD 909: Deletion of the aroC, aroD, and htrA genes with sustained expression of the Vi polysaccharide	Oral	Pre-clinical	50, 72
SII-Typhoid Conjugate Vaccine	Serum Institute of India	Vi-TT combined with O:2-TT	Intra-muscular	Phase I ongoing	50, 72
Vi-CRM197 with O:2-CRM197	GSK Vaccines Institute for Global Health & Biological E Ltd	O antigen from *S. Paratyphi A* chemically conjugated with CRM197, alongside Vi polysaccharide also linked to CRM197	Intra-muscular	Phase I ongoing	50, 72
O:2 DT with Vi-DT	International Vaccine Institute and Lanzhou Institutes of Biological Products	O antigen from *S. Paratyphi A* chemically conjugated with diphtheria toxoid, and Vi polysaccharide also conjugated with diphtheria toxoid	Intra-muscular	Pre-Clinical	72
The multiple antigen presenting system (Vi MAPS)+ O:2 MAPS	Boston Children's Hospital	polysaccharide-protein complex combined with pneumococcal fusion protein as the carrier	Intra-muscular	Pre-clinical	69,72
Vi+ SPA OMV	University of Cambridge	Genetically modified GMMA expressing Vi-PS	Intra-muscular	Pre-clinical	70, 71

CRM, cross-reacting material 197; GMMA, generalized modules for membrane antigens; OMV, outer-membrane vesicle

Supplementary Table II. Comparative insights to our candidate vaccines

S. no.	Parameters	Vaccine Candidates					
		Vi-TT		OSP-T2544	Vi-T2544		CTB-T2544
1.	Delivery Route	Subcutaneous (SC)/ intramuscular (IM)		Subcutaneous	Subcutaneous (SC)/ intramuscular (IM)		Intranasal
2.	Dose	25 µg of Vi		8 µg O-SP and 24 µg rT2544 in the conjugate	25 µg Vi and 29 µg rT2544 in the conjugate		60 µg
3.	Repeated booster	3 doses at 14 days interval		3 doses at 14 days interval	3 doses at 14 days interval		3 doses at 12 days interval
4.	Immunogenicity	SC	IM	SC	SC	IM	IN
	Serum antibody (IgG titer value)	Anti Vi=3200	Anti Vi=1280	Anti OSP=25600 Anti-rT2544=51200	Anti-rT2544 IgG 12800	Anti Vi=6400 Anti-rT2544=51200	Anti-rT2544 IgG 12800
	Mucosal antibody(IgA titer value)	Anti Vi, Serum = 320 Intestinal Lavage=160 Fecal extract=80	Anti Vi, Serum = 160 Intestinal Lavage=80 Fecal extract=80	Anti OSP, Serum = 640 Intestinal Lavage=320 Fecal extract=160	Anti- rT2544 Serum =2560 Intestinal Lavage = 2560 Fecal extract =1280	Anti Vi, Serum = 640 Intestinal Lavage=160 Fecal extract=160	Anti- rT2544 Serum =2560 Intestinal Lavage = 2560 Fecal extract =1280
	Antibody secreting cells (ASCs) (% of rT2544 specific ASCs out of total ASCs)	NA		NA	NA		IgA ASCs- Spleen and MLN =30% PP= 44% IgG ASCs- Spleen and MLN =12% PP= 22%
5.	Functional potency of antibodies						
	Adhesion Inhibition capacity	NA		NA	NA		Yes
	Opsano-phagocytic ability	NA		NA	NA		Yes
	Motility	No		Yes	No		Yes
	Bactericidal activity	SC On 38d *S.* Typhi= 800 *S.* Paratyphi A=NA On 140d *S.* Typhi= 1600 *S.* Paratyphi A=N IM (120d) *S.* Typhi= 400 *S.* Paratyphi A=NA (172d) *S.* Typhi= 800 *S.* Paratyphi A=NA		On 38d *S.* Typhi= 1600 *S.* Paratyphi A =12800 *S.* Typhimurim=6400 *S.* Enteritidis=1600 On 120d *S.* Typhi= 3200 *S.* Paratyphi A =25600 *S.* Typhimurim=12800 *S.* Enteritidis=3200	SC On 38d *S.* Typhi= 3200 *S.* Paratyphi A =12800 On 140d *S.* Typhi= 6400 *S.* Paratyphi A =25600 IM (38d) *S.* Typhi= 1600 *S.* Paratyphi A=6400 (140d) *S.* Typhi= 3200 *S.* Paratyphi A=12800		NA

Contd...

S. no.	Parameters	Vaccine Candidates			
		Vi-TT	OSP-T2544	Vi-T2544	CTB-T2544
6.	Cell mediated immune response	Th1/Th2	Th1/Th2	Th1/Th2	Th1/Th2/ Th17
	Cytokines responded	Th1 (IFNg) andTh2 (IL-4, IL-10)	Th1 (IFNg, TNF-a) andTh2 (IL-4, IL-10, IL-6)	Th1 (IFNg) and Th2 (IL-4, IL-10)	Th1 (IL-12, IFN-γ), Th2 (IL-4, IL-5, IL-10) and IL-17A
	Cytotoxic T lymphocytic assay	NA	NA	NA	NA
	Follicular Helper T cell (T_{FH}) (CXCR5+, PD-1+ , CD4+ cells)	NA	NA	NA	6% T_{FH}
7.	Gut homing memory response	NA	NA	NA	Yes, B & T lymphocytes expressing gut homing markers
8.	Memory B response	Observed till 140 days in mice	Observed till 120 days in mice	Observed till 140 days in mice	Observed till 120 days in mice
9.	Memory T cell subsets	Observed till 130 days in mice	Observed till 110 days in mice	Observed till 130 days in mice	Observed till 120 days in mice
10.	CD4+ T cells secreting IFNγ	Observed till 130 days in mice	Yes, observed till 110 days in mice.	Observed till 130 days in mice	Yes, Observed till 120 days in mice
11.	Protection in mice	60% for *S.* Typhi (SC) 40% for *S.* Typhi (IM)	75% for *S.* Typhi	90% for *S.* Typhi (SC) 77% for *S.* Typhi (IM)	70% for *S.* Typhi
		No protection was observed	77% *S.* Paratyphi A	80% for *S.* Paratyphi A (SC) 70% for *S.* Paratyphi A (IM)	80% for *S.* Paratyphi A
			80% *S. typhimurium*		
12.	Cross protection	NA	55-60% *S. enteritidis*	NA	Cholera toxin
13.	Current status	Commercially available	Preclinical and in negotiation for advancement of technology transfer	Preclinical	Preclinical
14.	Reference	Unpublished	[90]	Unpublished	[81]

Vi-TT, Vi conjugated to tetatnus toxoid; OSP-T2544, O polysaccharide conjugated to T2544; Vi-T2544, Vi conjugated polysaccharide linked to T2544; CTB-T2544, cholera toxin B genetically fused to T2544; Ig, immunoglobulin; NA, Not available