New Advances in Typhoid Fever Vaccination Strategies

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1 Introduction

Salmonella belong to the group of Enterobacteriaceae that are aerobic, gram-negative rods and approximately 1–3 μm × 0.5 μm in size [1, 2]. Currently there are approximately 2,400 pathogenic species of *salmonella*. *Salmonella* was first identified in 1880 by Eberth from the mesenteric nodes and spleen of a patient dying from typhoid fever [3, 4]. Later in 1884 Gaffky was able to isolate the bacillus. A year later Salmon and Smith described a bacillus that is now known to be *S.* Choleraesuis, the first bacteria that affects both human and animals [5]. *Salmonella* possess a flagellar antigen (H), somatic (O), and a surface antigen Vi. *Salmonella* are divided into two subspecies of *S. enterica* and *S. bongori*. *S. bongori* contains 8 serovars and *S. enterica* contains the other approximately 2,300 serovars that are divided into 6 subspecies based on flagellar H antigen. *Salmonella* nomenclature has undergone many changes [6]. Serotypes of *Salmonella* are recognized using the technique recommended in the Kauffman–White scheme.

Only few *Salmonella* serovars have been identified to cause disease in animals [7]. *Salmonella* subspecies *enterica* serovar Typhi is the most common cause of infection in humans and serologically is placed in *Salmonella* group D due to O antigens 9 and 12 [8]. The genetic makeup of the organism has not shown variation geographically and is stable with a few exceptions of isolates from Indonesia that have slightly different flagellar antigens. *S.* Typhi expresses a polysaccharide capsule Vi (virulence antigen) on its surface and is highly stable serologically compared to other *Salmonella* serotypes [9]. Presence of Vi prevents the binding of O antigen to the O antibody and thus enables the pathogenesis of the organism. Clinical severity of typhoid fever is a result of the Vi antigen that increases the infectivity [10]. However, Vi-negative strains have also been identified; therefore, Vi presence is not essential for *S.* Typhi-related typhoid fever. In vitro studies have shown that the Vi antigen of *S.* Typhi has anti-opsonic and antiphagocytic characteristic that reduces

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the level of secretion of *Salmonella* serovar Typhi-induced tumor necrosis factor alpha (a marker of activation) by human macrophages and increases the level of resistance of the organism to oxidative killing [8].

2 Typhoid Fever Epidemiology

A recent analysis estimated that there are 21 million typhoid fever cases per year and 216,000 deaths [11]. An earlier WHO estimate of the global typhoid disease burden based on a study from 1984 indicated around 17 million cases and approximately 500,000–600,000 deaths per year [11, 12]. Recent analysis assumes an average case fatality rate (CFR) of only 1%, which is at the low end of most estimates in the literature. Typhoid fever is considered endemic in most of the developing world. An estimated 90% of typhoid-related deaths occur in Asia [11, 13].

The recent burden of disease analysis was based on data derived from selected studies in a total of only 10 developing countries that included only one from sub-Saharan Africa (South Africa). High incidence rates of typhoid have been documented for south and Southeast Asia, but arbitrary estimates were made for many regions of the developing world that lacked any data, especially Africa. The paucity of reliable incidence data from most developing countries reflects the fact that laboratories capable of bacteriologic confirmation are lacking in much of the developing world [13]. As well as typhoid fever being endemic, the disease has also appeared as epidemic forms in central Asia, Africa, and south Asia [14].

The incidence of typhoid fever may vary considerably not only between, but also within, countries [15] (Fig. 1). In some countries, evidence suggests that residents of

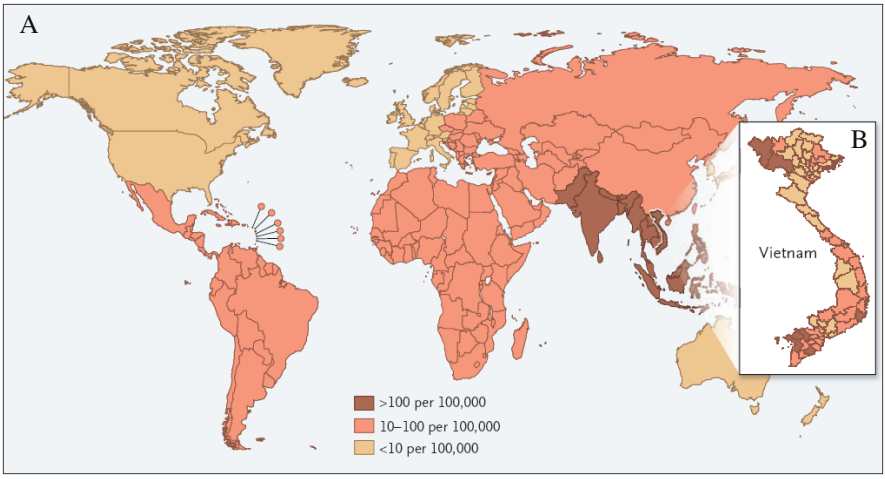

Fig. 1 Estimated distribution of typhoid fever burden in 2000 (a) and the geographic differences of typhoid fever incidence in Vietnam (b) [15]

poor urban areas are at considerably higher risk than rural dwellers. Until recently typhoid was considered as a disease of school-aged children. More recent systematic population-based studies from India, Bangladesh, and Pakistan have confirmed that the incidence is higher in young children [16–19].

Typhoid fever is a waterborne disease transmitted by the ingestion of food or water contaminated with excreta of patients and asymptomatic carriers and is therefore most common in areas with poor water and sanitation systems and practices. Common sources include polluted water and contaminated food (e.g., milk products), often eaten outside of the home and handled by infected persons. Other risk factors for increased transmission include recent typhoid fever in the household, a lack of toilet in the household, drinking unboiled water, not using soap for hand washing, and sharing food from the same plates as others [20, 21].

3 Typhoid Fever: Clinical Presentation and Outcome

Typhoid fever caused by *Salmonella* Typhi is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissue, and gall bladder. The clinical presentation of the disease varies from high-grade fever to more systemic involvement of nervous system [22]. Typhoid is often confused with other acute febrile illnesses until it persists for more than 3 days and does not respond to symptomatic treatment or first-line antimicrobial therapy [23]. Typhoid was recognized as a distinct disease in the earlier quarter of nineteenth century and soon gained significance as a serious health problem due to its ability to spread quickly in populations, especially those living collectively such as army soldiers and in dormitories [2, 20, 24].

Presentation of typhoid fever varies ranging from mild fever to more severe forms such as toxic shock. Symptoms include sustained high-grade fever (~104°F), profuse sweating, altered bowel habits from constipation in adults to diarrhea in children, malaise, myalgia, a dry cough resembling bronchitis, anorexia, nausea, and in some cases non-bloody diarrhea. If fever lasts for more than 5 days, a rash of flat, rose-colored spot may appear. The incubation period for a non-complicated case of typhoid fever is 10–14 days. Malaise and lethargy can continue for a couple of months even when the disease may have resolved. If left untreated, typhoid fever progresses through the four stages, each lasting approximately 1 week. In the first week, there is a slowly rising temperature with relative bradycardia, malaise, headache, and cough. In some cases bleeding from nose (epistaxis) and abdominal pain may also occur. The number of circulating white blood cells decreases with eosinopenia and relative lymphocytosis; blood cultures are positive for *Salmonella* Typhi, while Widal is negative in the first week [25, 26].

In the second week, fever has a plateau of around 104°F and heart rate is slow with a thready pulse. Delirium is frequent and calm, but sometimes agitated. Rose spots appear on the lower chest and abdomen in around 30% of patients. Abdominal symptoms become more obvious with pain in the right lower quadrant. Diarrhea with a frequency of six to eight stools per day may occur during this time; however,

constipation is also frequent. The spleen and liver become palpable and tender at this time. Elevation of transaminases can be seen on liver enzyme tests. Anti O and Anti H on Widal are strongly positive; blood culture may also be positive depending on the quantity of blood taken from the patient. In the third week of fever, complications appear including intestinal hemorrhage, encephalitis, metastatic abscess, cholecystitis, endocarditis, and osteitis. Overall 10–15% of typhoid fever cases develop complications. Intestinal perforation may occur in 1–3% of cases leading to peritonitis and ultimately to death if proper surgical intervention is not undertaken. In the fourth week fever is still high and oscillates very little. The patient has delirium due to dehydration. Other complications include disseminated intravascular coagulation that may lead to early death. Pneumonia is more common in children than in adults. Some of the rare outcomes reported are hepatic, splenic, and bone marrow granulomas; splenic and liver abscesses; pleural effusion; phagocytic syndrome; pseudotumor cerebri; hemolytic endocarditis and pericarditis. Arrhythmias or cardiogenic shock are manifestations of toxic myocarditis with fatty infiltration of the heart [1, 2, 24].

Hospitalization rates of typhoid fever cases vary from 10 to 40%, while the rest either self-medicate or are treated on an outpatient basis [16]. Population-based studies have reported variation in hospitalization rates. In settings where early treatment was provided due to extensive and systematic surveillance, it was possible to treat typhoid early. On the other hand patients who followed the regular health system mechanism had higher rates of hospitalization and complication. The average length of hospital stay ranges from 10 to 15 days.

Following recovery, convalescing patients may continue to excrete *S*. Typhi in the feces for almost 3 months. One to four percent of cases become long-term carriers, excreting the organism for more than 1 year. Most carriers are asymptomatic. The average case fatality rate is less than 1%, but this is variable among the endemic countries, with Pakistan and Vietnam having a case fatality rate of less than 2% and Indonesia and Papua New Guinea as high as 30–50%. Young children have been found to be at a higher risk of severe typhoid. Case fatality rates have been found to be 10 times higher in children younger than 4 years compared to older children. The most significant contributor to a poor outcome is a delay in the initiation of an effective antibiotic treatment. In untreated cases, fatality can go as high as 10–20%. The gall bladder carriage rate is 1–5% of the survivors of typhoid infection. Carrier status also increases the chances of hepatobiliary cancers [24, 27–30].

3.1 Diagnosis

Following ingestion of the *Salmonella* pathogen, there is an asymptomatic period. The incubation period for typhoid fever is 7–14 days and is influenced by the dose of the inoculum. Secondary bacteremia follows infection and coincides with the onset of symptoms such as high-grade fever and malaise. Other symptoms and signs that may help in the clinical diagnosis are loss of appetite, abdominal discomfort, headache, and severe myalgia. A coated tongue, tender abdomen, hepatomegaly,

and splenomegaly are also common. Delirium, confusion, and convulsions may also occur in children less than 5 years. As a result of bacterial dissemination throughout the body, the patient may present with systemic involvement such as respiratory, neurological, and abdominal illnesses.

The diagnosis of typhoid fever in endemic settings is mostly clinical and relates to the clinical experience of the attending physician. There have been repeated and regular attempts to establish diagnostic criteria that combine clinical presentation and laboratory investigations. Such attempts have not resulted so far in the development of a diagnostic technique that will help overcome current diagnostic challenge. Despite reservations about the sensitivity, specificity, and predictive value of Widal, it is the most common laboratory method used for diagnosis of typhoid. Widal detects antibodies that are also cross-reactive with other Enterobacteriaceae. In typhoid patients, antibodies only appear in the second week; therefore, usefulness of the test is limited in the initial stages of the disease [31]. Other serological tests such as Tubex and Typhidot have not shown promising results. The gold standard for the diagnosis of typhoid is isolation of the bacteria from blood and/or bone marrow. Bone marrow cultures have higher sensitivity compared to blood culture. The bone marrow culture is positive for 80–95%. In cases where patients have been treated with antimicrobials, the bone marrow culture may still lead to *S. typhi* isolation. Blood culture is positive 60–80% of the time but the yield varies with the quantity of blood taken [23, 32–35].

3.2 Management

Lack of simple, accessible cost-effective tools for accurate diagnosis of typhoid fever results in delayed diagnosis and failure to adequately treat the disease. These factors in turn contribute to the high emergence of severe form of the disease in endemic settings. In initial stages, the disease is either treated at home or by informal health sector. Improper diagnosis leads to inappropriate management and resultant increase in severity of the disease ultimately leading to hospitalization and fatal outcomes. Careful assessment of fever cases is recommended. In cases where fever is more than 5 days, laboratory investigation such as blood culture is advised. However, clinical symptoms and signs should be correlated with laboratory findings. In cases where either the provisional diagnosis is typhoid fever or there is serological or bacteriological evidence of disease, first-line antimicrobial therapy should be initiated. Third-generation cephalosporins are the most effective treatment for typhoid fever with cure rates of 90–98% [27].

4 Control Strategies

Similar to other diseases spread by the fecal–oral route, typhoid fever predominates in areas with inadequate water and sanitation systems and/or poor hygienic practices. Typhoid was effectively eliminated in developed countries mainly through large-scale development of water treatment (e.g., chlorination), construction of deep

wells, and piped water and sewerage systems. Impact of safe drinking water and adequate sanitation on diarrheal diseases has also been demonstrated in northeast Brazil where a 22% reduction in diarrheal diseases was evident after an expenditure of nearly 900 million dollars on infrastructure development. Infrastructure development for provision of safe water and proper sanitation is costly to build for many developing country government budgets. Considering most of the typhoid fever cases occur in urban slums of Asian cities, diversion of development budgets seems unrealistic in near future [36–38].

In lieu of the existing situations, alternative short-term interventions are recommended for the reduction of disease burden in these areas. These interventions include intensive hygiene education for hand washing using soap, discouraging open defecation especially by children, and the proper disposal of garbage and feces. There is evidence that such interventions have been effective in the control of enteropathogens at small scale. The practicality of such interventions at large scale has still not been answered systematically [39–42].

In the existing circumstances, a typhoid fever vaccination program may provide a short-term alternative strategy coupled with a continuous advocacy for development of infrastructure for safe water provision and clean and hygienic sanitation. There is evidence that immunization can virtually eliminate typhoid fever in a relatively short period of time, especially when targeted toward high-risk age groups and geographic areas. Due to the reduction in the price of the vaccine, it is now becoming more affordable to countries with high burden of typhoid fever. In order to make a typhoid fever vaccination program more effective, it must be introduced as a typhoid fever control program that should have other components such as hygiene education messages, sanitation improvements (e.g., latrines), and improved water supply and quality measures [19, 37, 43–47].

4.1 Antimicrobial Resistance

Increasing resistance to available antimicrobials is another challenge for typhoid fever control. Outbreaks of *S.* Typhi strains resistant to chloramphenicol first appeared in the 1970s in several parts of the world. As new drugs such as ampicillin and co-trimoxazole became available, resistance against these drugs also emerged. Outbreaks of multi-drug resistance (MDR), defined as resistance to first-line antibiotics, were first reported in the late 1980s in south Asia and the Middle East that later spread to east Asia and Africa. In Vietnam, 86% of all isolates were found to be multi-drug resistant. MDR typhoid has been associated with more severe illness and higher rates of complications and deaths, especially in children under 2 years of age. The emergence of multi-drug resistance *S.* Typhi strains has led to the widespread use of fluoroquinolones, such as ciprofloxacin and ofloxacin. However, outbreaks of nalidixic acid-resistant typhoid (called NARST) started to occur in Vietnam and Tajikistan in the early 1990s and then spread to Pakistan and India [32]. Nalidixic acid-resistant typhoid cases respond less well to fluoroquinolones, exhibiting more prolonged fever than sensitive cases, and, in one study, a 10-fold higher rate of

post-treatment stool carriage was observed compared to sensitive cases (20% vs. 1.8%), increasing their potential to infect others. Cases of full-blown resistance to ciprofloxacin have also reported from Pakistan and India [33, 48–53].

More recent data from population-based studies confirm that multi-drug and nalidixic acid resistance is a serious problem in south and southeast Asia [14]. Sixty-seven percent of isolates tested in Karachi, 22% in Hue, and 7% in Kolkata were multi-drug resistant, and high rates of nalidixic acid resistance were found in all three sites – 59% in Karachi, 58% in Kolkata, and 44% in Hue. Two isolates in the India site (1.6%) were found to be ciprofloxacin resistant. On the other hand, no drug resistance was found in the Indonesian and Chinese sites (Table 1). The increase in the resistance to available antibiotics may result to increase in the fever duration, decrease in management options, and an economic burden on the families. The often non-specific symptoms of typhoid fever can make the clinical diagnosis difficult and it can be confused with malaria, dengue fever, influenza, and other febrile illnesses. Confirmed diagnosis requires isolating *S.* Typhi in the laboratory through blood cultures, bile-stained duodenal fluid culture, or occasionally through bone marrow culture. Unfortunately, such invasive tests are not conducted for the majority of patients in developing countries, especially those treated in non-hospital settings [14, 24, 54].

Table 1 Antibiotic resistance among *Salmonella typhi* isolates from five Asian study sites in the DOMI program

	Hechi, China	Kolkata, India	N. Jakarta, Indonesia	Karachi, Pakistan	Hue, Vietnam
Total number of isolates tested	15	122	131	127	18
Antibiotic resistance (%)					
Chloramphenicol	0 (0%)	9 (7%)	0 (0%)	85 (67%)	6 (33%)
Ampicillin	0 (0%)	9 (7%)	0 (0%)	84 (66%)	6 (33%)
TMP–SMX[a]	0 (0%)	11 (9%)	0 (0%)	84 (66%)	4 (22%)
MDR[b]	0 (0%)	9 (7%)	0 (0%)	83 (65%)	4 (22%)
Ciprofloxacin	0 (0%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nalidixic acid	0 (0%)	69 (55%)	0 (0%)	75 (59%)	8 (44%)

Source: DOMI program; data from [14]
[a]TMP–SMX, trimethoprim–sulfamethoxazole
[b]MDR, multi-drug resistant (i.e., resistant to chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole)

4.2 Vaccination

The use of new-generation antimicrobials to manage increasing resistance has increased the cost of treatment. The cost of illness, due to antimicrobial-resistant typhoid, is on average nearly four times greater than those who responded well to

Table 2 Total cost of blood culture-confirmed typhoid fever illness by the type of patient and study site (US $2005) in the DOMI program

Type of patients and costs	Hechi, China	Delhi, India	Kolkata, India[a]	N. Jakarta, Indonesia	Karachi, Pakistan[b]	Hue, Vietnam
Sample size	58	98	79	107	66	16
Ages (years) included in surveillance	5–60	All ages	All ages	All ages	2–15	5–18
Total costs for hospitalized patients	215	820	129	432	210	157
Costs for outpatients	67	95	13	57	38	38
Hospitalization rate (%)	40	12	2	20	10[a]	28
Weighted average costs (hospitalized and outpatient)						
Private costs	126	79	11	106	53	38
Direct	101	43	6	61	45	33
Indirect	26	36	5	45	9	5
Public costs	0	101	4	26	2	33
Total weighted average costs	$126	$182	$15	$132	$55	$71
Average household monthly income in sample (US $2005)	121	N/A	N/A	207	158	84
Percent of families that borrowed money for typhoid treatment (%)	14	N/A	49	24	N/A	18

Sources: Poulos C et al. Cost of illness due to typhoid fever in study sites in five Asian countries *Unpublished* (*Manuscript submitted*)

[a]Bahl et al. [55]

[b]Results from Karachi are based on local expert opinion and reflect the costs of disease in children 2- to 15-year-old

the first-line antimicrobial treatment (Table 2). Typhoid fever can have a devastating financial impact on families in several of these largely poor communities as the majority of costs of illness are private costs equivalent of 1.6 and 1.2 months of an average household income. These findings of disease burden from various parts of the world have important implications for typhoid fever immunization strategies at the country level, as they suggest that in many countries, vaccination in geographically targeted, high-risk populations, rather than universal immunization, will be potentially the most cost-effective means of controlling the disease [55].

Typhoid vaccine use for prevention of disease dates back to 1896 with the inoculation of heat-inactivated vaccine. This was the first bacterial vaccine to be widely used in humans. The vaccine was obtained by inactivating the virulent microorganisms with heat or chemicals. The associated adverse effects after administration of killed whole-cell vaccine restricted its wider public health use. The adverse events included fever (6–30%), malaise, local reaction (35%), and headache (10%). During the World Health Organization-sponsored trials, the efficacy of the vaccine was 51–66%, but it was highly reactogenic. Among vaccinees 25% had systemic and local reactions post-vaccination. It is believed that during the process of vaccine

production, destruction of some heat-labile antigens resulted in the low efficacy and associated adverse effects. Irrespective of being highly reactogenic, the vaccine was widely used in the military in the early twentieth century due to high reporting of typhoid fever in the sick reports of the English and American armies. The vaccine was shown to reduce typhoid incidence by more than 90% from the time before vaccine introduction. Similarly the Belgian government conducted mass vaccination of the civilian population during the First World War in 1915. More recent examples of use of the killed WC vaccine are in schools and high-risk population in Thailand in the 1960s and early 1970s. A drop in the incidence of typhoid fever was noticed after the introduction of the vaccine. Similarly Cambodia used the vaccine during an outbreak; however, adverse events resulted in the dropping of the fourth dose [7, 56, 57].

In early twentieth century inactivated oral vaccines (acetone-inactivated vaccine and formalin-inactivated vaccine) were used to assess local immunity. These oral inactivated vaccines were evaluated in volunteers and field studies in the 1960s and 1970s. These vaccines could not make it to efficacy assessment and are no longer under consideration for production. The two new-generation typhoid vaccines that are currently internationally licensed and available are the injectable Vi polysaccharide vaccine and the oral, live-attenuated Ty21a vaccine [58–61].

4.2.1 Ty21a

Ty21a is an orally administered, live-attenuated Ty2 strain of *S.* Typhi in which multiple genes have been chemically mutated, including those responsible for the production of Vi. The vaccine was developed in the 1970s and first licensed in 1989, but initially used only in developed countries (Table 3). This lyophilized vaccine is currently available in two formulations. The enteric-coated capsules given in three to four doses and a liquid suspension consisting of the vaccine in one sachet and a buffer in another are combined with water before administration. The liquid formulation is given in three doses. For both formulations, the doses are administered every other day (e.g., over a 5-day period). The vaccine is licensed for use in persons 6 years and older. While the capsules are often used for travelers to developing countries, the liquid formulation is the one most likely to be used by public health programs in developing countries. The vaccine requires a cold chain (at 2–8°C) and survives for approximately 14 days at 25°C. Ty21a vaccine has been shown to be well tolerated and to have low rates of adverse events. In three double-blinded, randomized controlled efficacy trials in Chile and Indonesia involving approximately 550,000 school children, reactogenicity of the Ty21a vaccine was assessed through active surveillance. The rates of side effects (diarrhea, vomiting, fever, and rash) in the vaccinated groups were not found to be significantly greater than those in the control groups for both the enteric-coated capsule and liquid formulations. In large-scale field trials in children in Egypt, Chile, and Indonesia, Ty21a was found to have protective efficacy rates against blood culture-confirmed typhoid fever of 33–67% for the enteric-coated capsules and 53–96% for the liquid formulation (53–78% for the currently licensed liquid formulation) after 3 years of follow-up, when each was

Table 3 Description of efficacy and effectiveness trials of Ty21a oral typhoid vaccine

Study (Year)	Reference	Formulation	Number of study subjects	Ages (years)	Follow-up period	PE for blood culture-confirmed typhoid (95% CIs)	Incidence rate in control group (per 100,000)
Alexandria, Egypt (1978–1980)	Wahdan et al. [83]	Liquid given with tablet of $NaHCO_3$	32,388	6–7	36 months	96% (77–99%)	50
Area Occidente, Santiago, Chile (1983–1986)	Levine et al. [84]	Three doses of enteric-coated capsules given (1–2 days between doses)	140,000	6–19	36 months 7 years	67% (47–79%) 62%	110
Area Sur Oriente, Santiago, Chile (1986)	Black et al. [85]	Three doses of enteric-coated capsules (1–2 days between doses)	81,321	6–19	3 years	33% (0–57%)	100
		Three doses liquid suspension (1–2 days between doses)			3 years 5 years	77% 78%	
Sumatra, Indonesia (1986–1989)	Simanjuntak et al. [63]	Three doses of enteric-coated capsules (7 days between doses)	20,543	3–44	30 months	42% (23–57%)	810
		Three doses liquid suspension (7 days between doses)				53% (36–66%)	

given in three doses every other day (except in Indonesia, where dosing occurred every 7 days). The vaccine appeared to be more efficacious in areas with lower incidence of typhoid (Egypt, Chile) than in hyper-endemic areas, such as Indonesia. Ty21a is therefore considered to provide protection for at least 5–7 years. Large-scale vaccination with Ty21a also appeared to confer herd protection in Chile. These data suggest that the systematic application of live oral typhoid vaccine can notably reduce the incidence of the disease in endemic areas [12, 62–65].

4.2.2 Vi Capsular Polysaccharide

Vi is a subunit vaccine consisting of the purified Vi ("virulent") polysaccharide outer capsule of the Ty2 strain of *S.* Typhi. The vaccine is administered subcutaneously or intramuscularly as a single dose of 25 μg. It was first developed in the 1970s and further developed for large-scale manufacture at the US NIH, in collaboration with Pasteur-Merieux-Connaught. First licensed in the USA in 1994, the vaccine is in the public domain and is now being produced by several multi-national and developing country manufacturers. Like other T-independent purified polysaccharide vaccines, Vi does not elicit adequate immune responses in children less than 2 years of age, and thus is licensed for use in persons 2 years and older. The vaccine is highly heat stable and is able to retain its physicochemical characteristics for 6 months at 37°C and for 2 years at 22°C (room temperature). Vi vaccines have been extensively tested in humans and demonstrate a strong safety profile (Table 4). No serious adverse events and minimum side effects were associated with Vi vaccination in large field trials. In a recent multi-center study of Vi effectiveness, the vaccine showed safe and with minimal side effects. There is no booster effect of Vi vaccine [8, 10, 66–72].

4.2.3 New Vaccines in Pipeline

The low-efficacy estimates, inability to confer lifelong immunity, and difficulties in administration through regular and routine public health programs have limited the use of available typhoid vaccines. Therefore, a search for new improved vaccine is on the agenda in the vaccine field. There have been attempts to produce conjugate typhoid vaccines in both oral and parenteral forms. The aim of a conjugate vaccine is the production of T-cell-dependent immunity where the serum antibody response can be boosted and results in long-term immunity. Tetanus and diphtheria toxoid, cholera toxin, cholera toxin B subunit of recombinant exotoxin A of *Pseudomonas aeruginosa* are being tested for conjugation to Vi. An earlier Vi conjugate vaccine did not produce significant results due to the high Vi volume. Recent advances in the conjugation of Vi to a carrier protein have led to significant antibody responses in adults and children in endemic settings. A similar approach has been adopted for oral vaccines using recombinant techniques. The aim is to have a vaccine that will be single dose and will induce sufficient immunity to protect the population for life. However, to have such a vaccine seems overambitious at this moment. Both Vi conjugate vaccines, designed to be effective in infants, and new oral live vaccines, designed to be highly immunogenic in a single dose, are currently in

Table 4 Description of Vi Polysaccharide vaccine efficacy trials

Study (year)	Reference	Formulation	Number of study subjects	Ages (years)	Follow-up period	PE for blood culture-confirmed typhoid (95% CIs)	Incidence rate in control group (per 100,000)
Kathmandu Valley, Nepal (1986–1988)	Acharya et al. [68]	One dose of Vi (25 μg)	6,907	5–44	17 months	72% (42–86%)	926
E. Transvaal, South Africa (1985–1988)	Klugman et al. [69]	One dose of Vi (25 μg)	11,384	6–14	21 months 36 months	64% (36–79%) 55%	773
Quan County, Guangxi Province, China (1995–1997)	Yang et al. [86]	One dose of locally produced Vi (30 μg)	131,271	3–50 (92% school age)	19 months	69% (28–87%) (72% in school children)	63–78

development. A prototype Vi conjugate vaccine was found to be highly efficacious (91%) in Vietnamese toddlers for at least 4 years and serum antibody responses suggest that it can protect for at least 10 years in persons 5 years and older. Several groups are now developing Vi-diphtheria toxoid (DT) conjugate vaccines, with the goal of transferring technology to appropriate developing country producers, so that low-cost typhoid conjugate vaccines can ultimately be incorporated into the infant EPI schedule for high-risk populations. A number of improved live oral vaccines are currently in clinical trials. However, all of these newer generation typhoid vaccines are still several years away from being licensed and available on the market. The future promise of these vaccines should not preclude the more immediate use of currently available new-generation vaccines in endemic populations.

4.3 Perceived Risk of Disease and Vaccination Acceptance

Research suggests that vaccine acceptance or demand can be influenced by the perceived prevalence of the disease in the community, as well as by beliefs regarding the severity of the disease, the risk of its striking one's household, attitudes toward vaccination in general and perceived benefits and risks of specific vaccines. Among other factors, knowledge of and experience with the disease are also important factors. Communities also exhibit a strong understanding of how common the disease is in their communities. There is a strong correlation between actual incidence and perceptions of typhoid being a "common" or "very common" disease in their community. High-risk communities also tend to have good knowledge of how to prevent typhoid fever. There has been interest from high-risk population and demand for new-generation typhoid vaccines (Table 5). The findings from socio-behavioral studies also highlight the demand for typhoid vaccine in areas where incidence of typhoid fever was not high [47, 73, 74].

4.4 The Market (Vaccine Demand and Supply)

According to preliminary estimates, the potential demand for a typhoid vaccine was calculated for 30 countries in regions considered to have high typhoid incidence (>100/100,000/year). The estimated number of doses required each year was approximately 136 million. Given that there are several high-quality producers of Vi the issue of supply of Vi vaccine does not appear to be a problem with manufacturers being able to meet an increased demand for new-generation typhoid vaccines created by their introduction into public health program in endemic countries.

In the years since the WHO recommendation, several developing country manufacturers have acquired the technology to produce Vi. This proliferation of Vi producers has been facilitated by technology transfer from the US National Institutes of Health (NIH) to several companies, the lack of patent protection,

Table 5 Results of the DOMI socio-behavioral studies on population knowledge, perceptions, and beliefs in five Asian sites

Data	Hechi, China	Kolkata, India	N. Jakarta, Indonesia	Karachi, Pakistan	Hue, Vietnam
Annual typhoid incidence in 5- to 15-year-olds (per 100,000)	29	494	180	413	24
Percent of respondents who have heard of or are familiar with typhoid fever (%)	73	93	N/A	86	77
Households who report past experience with typhoid fever in the household (%)	14	37	48	31	2
Percent who believe that typhoid fever is "common" or "very common" in community (%)	2.5	66	25	47	4
Percent who think the chances of household members getting typhoid fever are	(regarding children)				
• Very likely (%)	0.4	12	66	52	0
• Likely or somewhat likely (%)	9	64	24	6	48
Percent who consider typhoid fever in infants or children to be	N/A				
• Very serious (%)		56	22	58	57
• Serious (%)		38	67	37	39
• Total (%)		94	89	95	96
Percent who think that typhoid vaccines should be used in community (%)	50	93	97	95	N/A

as well as the relatively simple, low-cost production process involved. Two additional developing country producers are in the process of developing Vi vaccines, in collaboration with the International Vaccine Institute and the US NIH.

4.4.1 Vaccination Strategies

The typhoid fever burden estimates are available only from few countries globally. For countries where estimates are available, data come from small-scale population-based studies or conducted as part of surveillance for vaccine trials. Therefore, the introduction of typhoid vaccines for mass immunization is questioned. A more practical approach recommended by the WHO is to consider targeted introduction of the vaccine in national vaccination programs. The policy decision for typhoid vaccine uptake is largely dependent on the perception of typhoid endemicity in the country.

The estimates of clinical protection for typhoid fever have been consistent around 70% for at least 3 years across field trials. However, there has been little evidence on the effectiveness of the vaccine until recently. Results of the indirect protection in Kolkata suggest the actual impact of the vaccine is much higher than expected.

4.4.2 Determining Endemicity

The widespread use of antimicrobials has reduced complication rates of typhoid fever. However, population studies directed by hospital estimates have shown that high rates of typhoid incidence are captured once systemic surveillance is undertaken. Population-based studies are expensive and time-consuming. Therefore, in settings where typhoid fever is expected to be found, alternative methods can be adopted to assess disease burden. A rapid assessment of outpatient hospital visits, admissions, and outcome of fever episodes can provide approximate estimates about the most affected age group, geographic location, and socio-economic classes affected. Such data can then be used for typhoid fever advocacy, guiding control strategies and in determining the target population for vaccination. In endemic settings, focusing on the high-risk groups can be a cost-effective strategy. A vaccination campaign targeting high-risk populations such as school age children, food handlers will affect transmission of the pathogen and hence circulation in the environment. Such effects can reduce the burden of disease beyond controlled efficacy results for the vaccine.

A common source of typhoid spread in a high endemicity setting is food handlers. Unhygienic food is sold without control by street vendors. Considering the prevalence of typhoid fever, the chances that these food handlers will be carriers of typhoid qualify them as a priority group for vaccination. Typhoid incidence estimates from south Asia have shown that children of school age are at highest risk. Considering that 5% of cases become carriers after being infected, school-age children will have the highest rates of transmission and close interaction of children in school and sharing of food increases the risk of spread of *S*. Typhi infection from an infected child to other typhoid-susceptible children. Vaccinating school-aged children will also have a greater impact in disease reduction. School-based immunization in Thailand with the killed whole-cell vaccine in the 1980s provides lesson for countries with endemic typhoid [75].

5 Population Impact

Among the two vaccines available in the market, only Vi polysaccharide vaccine has been used at large scale in countries with a high burden of typhoid fever. The introduction of the vaccine resulted in a significant reduction of typhoid fever presenting to health clinics. However, a more scientific evaluation of the effect of the vaccine has not been done that could single out Vi vaccine use as the important factor in disease reduction.

5.1 Guangxi Province, China

Typhoid fever has been endemic in many southern provinces of China. An annual incidence rate of 113/100,000 in the general population was reported in Jiangsu

Province in 1988, and an average annual incidence of 53/100,000 between 1995 and 1999 in Hechi City in Guangxi Province. An immunization program using locally manufactured Vi vaccine was undertaken in these typhoid endemic areas in the 1980s. Initially the old- generation killed whole-cell vaccine and the new-generation oral live Ty21a vaccines were used. However, due to adverse events association with killed vaccine added with high cost and a difficult schedule of administration of Ty21a, the Ministry of Health switched to Vi polysaccharide vaccine in the program. Local production was a result of technology transfer to six institutes of biological products by the National Institute of Health United States.

Vi polysaccharide vaccine was introduced in the province of Guangxi in 1995; however, there are other provinces in China (provinces of Jiangsu, Hunan, Hubei, Yunnan, Guizhou, and Sichuan, and the cities of Beijing and Lanzhou) that have used Vi polysaccharide vaccine in a targeted program to reduce the burden of typhoid fever. Approximately 26 million doses of vaccine were given to school children and other high-risk groups such as food handlers. The most robust data on the impact of Vi polysaccharide vaccine on the incidence are available from the city of Guilin in Guangxi Province in southwest China from 1995 to 2006. Between 1995 and 2006, more than 1.3 million doses were administered to all target groups, peaking in 2000 and 2001. In all, 77% of the vaccine was given to students and 23% went to food handlers and residents of outbreak areas. Coverage rates have varied broadly from year to year, but have averaged 60–70% for students over the 11-year period and 80–85% for the other target groups [76].

The annual incidence of typhoid reported in the city averaged 57/100,000 in the student population and 42/100,000 in the non-student population from 1991 to 1994. Annual incidence rates of typhoid fever in Guilin from the National Notifiable Infectious Disease Reporting (NIDR) system showed the incidence declined to very low levels (0.2–4.5/100,000) in both the student and non-student population from 1995 to 2006 after vaccination [29]. Vaccine coverage ranged between 3 and 13% among the general population; between 15 and 74% among students. Approximately 3.5 million vaccines were provided to the target region in the specified period of time.

Typhoid vaccine is also recommended for use in outbreak settings in China. The recommendation is based on an effectiveness study of *S.* Typhi outbreak in China in 1999.

5.2 National Immunization Program, Vietnam

In 1997, the National Immunization Program (NIP), Vietnam, took the initiative of typhoid fever vaccination as a regular program. This decision was driven by the increase in the reporting of clinical typhoid fever and the rise in incidence of antibiotic resistance. Typhoid vaccination was limited to half of the 61 high incidence provinces. The vaccines were provided by the National Institute of Vaccines and Biological Substances (IVAC) to the NIP at price of approximately US $0.52 a dose. The typhoid vaccination program involved annual campaigns in which children

3–10 years of age were vaccinated with Vi polysaccharide in selected districts. Children as well as adults were vaccinated in districts with reported typhoid fever outbreaks. More than half a million doses of typhoid vaccine were given to 3- to 10-year-olds in the selected 30 provinces.

Review of the data from the NIP on the use of Vi polysaccharide vaccine in the northwestern region showed a clear decline in the incidence of typhoid fever from 97/100,000 persons per year in 1999 to less than 20/100,000 from 2006 after the introduction of Vi polysaccharide vaccine. Vaccine coverage in the general population ranged between 0.1 and 4%, but it was much higher among the targeted age group. A similar decline in the incidence of typhoid fever was seen not only from the southern Mekong delta region but also from other regions with medium typhoid incidences where Vi polysaccharide vaccine was introduced.

A meta-analysis of typhoid incidence data using prospective surveillance study results and the government's routine disease reports suggests that a targeted immunization strategy is appropriate to reduce the number of cases. An impact and financial analysis further suggests that Vi polysaccharide vaccination in these provinces would need to be more intensive (e.g., covering all districts in a given province) and systematic than the current program in order to have a significant impact on disease incidence in the country as a whole.

5.3 Delhi State, India

The State Government of Delhi, India, funded a typhoid vaccination program for 2- to 5-year-old children with Vi polysaccharide vaccine. The program represented the first public sector typhoid vaccination program in India since 1987 when the old whole-cell vaccine was discontinued due to its reactogenicity and due to the perception that typhoid fever was not a major cause of mortality. The impetus for Vi polysaccharide vaccine introduction was the emergence of multi-drug-resistant typhoid fever among children coming to the city's hospitals. The program targeted 2- to 5-year-old group children that are reported to be at a higher risk. The State Directorate of Family Welfare and the Delhi Municipal Corporation, which provides around 85% of the state's government health services, ran the program. The vaccines are purchased for US $0.53 from a local producer. Since the start of the program, approximately 1 million children have been vaccinated at a rate of 300,000–325,000 children per year. A systematic evaluation of the program is not available, and it is therefore not possible to assess the impact of vaccination on the incidence of culture confirmed or clinical typhoid in the age group and on the general population [77].

5.4 Disease of Most Impoverished (DOMI) Studies in South and SouthEast Asia

Through the DOMI Program, the Vi polysaccharide vaccine was used for a series of effectiveness trials in Asia. Project sites were established in five Asian countries:

Hechi, China; Kolkata, India; North Jakarta, Indonesia; Karachi, Pakistan; and Hue, Vietnam. Study sites were chosen in discussion with the local public health specialists on the basis of a high perceived burden of typhoid fever, absence of control programs against the disease, and willingness of the community to participate. The age groups selected were thought to be the likely targets for typhoid vaccination under a public health program. The projects were designed as a cluster randomized controlled effectiveness trial in all sites except for North Jakarta, which conducted a demonstration project to assess mass vaccination feasibility and safety. The project mimicked the way Vi polysaccharide vaccine might be delivered under public health conditions. In Indonesia and Vietnam, it was deemed most appropriate to target the school children at schools. In other sites, community-based vaccination was considered most appropriate. These decisions were made by the local public health experts and implemented for the projects.

Mass vaccinations were conducted in 2003 and 2004 in five sites, having more than 190,000 people vaccinated with Vi or a control agent. The program proved that very large mass vaccination campaigns are feasible and safe. The vaccination coverage in the target population was between 58 and 91%. The highest coverage rate (91%) was achieved in a school-based program in North Jakarta, Indonesia. The lowest coverage rate was observed in another school-based program in Hue, Vietnam. The community-based mass vaccination campaigns in China, India, and Pakistan had participation rates that ranged between 68 and 78%. Variations in the vaccination coverage might have been related to the different study designs [78–81].

A cluster randomized trial assessed the effectiveness of Vi polysaccharide vaccine through a cluster randomized effectiveness trial in Kolkata, India. 37,673 individuals of more than 2 years of age either received the Vi polysaccharide vaccine or the active control hepatitis A vaccine (Table 6). Protective effectiveness (PE) of Vi polysaccharide vaccine against typhoid fever was calculated to be 61% (95% CI: 41–75) 2 years after vaccination. The trial reported for the first time the Vi polysaccharide protection in children aged 2–5 years with a PE of 80% (95% CI: 53–91). The study reported no serious adverse event associated with the vaccine [82].

Table 6 Vi Polysaccharide effectiveness estimates from Kolkata, India [81]

Age group	Vaccine group		Total protection Vaccine protective effectiveness (95%CI)
	Vi	Hepatitis A	
2–4 years (cases/population)	5/1,097	27/1,095	82% (95%CI: 58%, 92%)
Incidence per 1,000 population	2.3	12.9	
5–14 years (cases/population)	21/4,282	54/4,584	59% (95%CI: 18%, 79%)
Incidence per 1000 population	2.5	6.1	
≥15 years (cases/population)	8/13,490	15/13,125	48% (95%CI: –44%, 81%)
Incidence per 1,000 population	0.3	0.6	

6 Conclusion

Typhoid fever in childhood differs significantly from clinical presentation from adults and case fatality rates are higher in children under 5 although complication rates are almost similar. There are few community-based studies that have looked specifically for typhoid fever. The global estimates of typhoid fever grossly under-report rates of complications and have no data on severity of disease and outcome. There are regional differences in presentation which may reflect differences in care-seeking patterns, health systems, and co-morbidities. Case fatality rates from sub-Saharan and North Africa were higher than Asia and those from central Asia. This may have resulted due to reporting during an outbreak period. There is no evidence that MDR typhoid is associated with consistently higher rates of complications and mortality. Recent emergence of nalidixic acid-resistant strains poses enormous challenges for developing countries with few affordable options for treating typhoid in public health settings. There is an urgent need for expanding the antibiotic pipeline for typhoid and innovative approaches including combination therapies, antibiotic cycling, and reverting to first-line therapy in sensitive cases.

Vi-PS vaccine, unless used at scale for mass vaccination, may not provide protection against typhoid among young children (under 5) in endemic areas. The last Vi-conjugate vaccine efficacy trial (with 89% protection) was over 10 years ago. There is need for alternative strategy of fast tracking Vi-conjugate vaccines in endemic areas, potentially in combination with other antigens (e.g., paratyphoid A).

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