Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Review Article | International Journal of Vaccine Research | Open Access

Typhoid Fever as a Challenge for Developing Countries and Elusive Diagnostic Approaches Available for the Enteric Fever

Arti Bharmoria[1*], Annpurna Shukla[2] and Kanika Sharma[2]

[1]*Department of Biotechnology, Sharda University, Greater Noida, Uttar Pradesh, India*

[2]*Central Drugs Laboratory, Central Research Institute, Kasauli, Himachal Pradesh, India*

Received: : 08 May, 2017; Accepted: 23 May, 2017; Published: 02 June, 2017

***Corresponding authors:** Arti Bharmoria, Department of Biotechnology, Sharda University, Greater Noida, Uttar Pradesh, India, E-mail: artibharmoria@gmail.com*

Abstract

Salmonella typhi is the causal organism for the typhoid fever. Typhoid disease is a problem in those developing countries where lack of sanitation, poor water supplies and exposure to unhygienic and polluted environment is a part of routine life of majority of population. *Salmonella typhi* expresses various virulence antigens like H-antigens, O-antigens and Vi antigens which plays vital role in the infection and pathogenesis of the bacteria. *Salmonella typhi* causes fever, nausea, headache, body fatigue and bowel related complications in the patients. This disease had been diagnosed by culture method, Widal test for past several decades. These diagnostic techniques are being replaced by the modern assays like ELISA, Dot blot ELISA, DipStick Assays, RT-PCR and other molecular assays. The evolution in diagnostic assays had occurred as the antibiotic resistance in the *Salmonella typhi* had shown an increase. The *Salmonella typhi* had been evolved into a multidrug resistant bacteria which need to be timely diagnosed to start the appropriate and correct treatment of typhoid to prevent further complications. This article imparts a brief glimpse on *Salmonella typhi*, typhoid fever and diagnostic assays available at clinical laboratories. There is a need to focus on the correct and rapid diagnostic methods which should be modified and made accessible to the clinical diagnostic laboratories.

Keywords: *Salmonella typhi;* Virulence Antigens; Typhoid; Vaccines; Diagnosis

Abbreviations

ELISA:Enzyme Linked Immunosorbent Assay; RT-PCR:Reverse Transcriptase Polymerase Chain Reaction; *S. typhi:Salmonella typhi;* DNA:Deoxyribo Nucleic Acid; NTS:*Non-typhoidal Salmonella;* LPS: Lipo Polysaccharide

Introduction

Enteric fever causes substantial illness and deaths in many parts of world, especially in poorer nations. 'Enteric fever' term is used to refer the typhoid and paratyphoid fever. Typhoid fever is one of the major bacterial infections worldwide especially in countries located south of the globe, which is caused by the infection of *Salmonella typhi.*

Typhoid fever is an acute generalized infection of the reticulo-endothelial system, intestinal lymphoid tissue and gall bladder caused by *Salmonella enteric serovar typhi*. Salmonellae are the member of the family Enterobacteriaceae. S*almonella enteric serovar typhi (S. typhi)* is a facultative intracellular pathogen that causes typhoid fever in humans [1]. The organisms are non-capsulated, non-sporulating, Gram-negative anaerobic bacilli, which have characteristic flagellar, somatic, and outer coat antigens [2]. Typhoid is restricted to human hosts and humans constitute the reservoir of infection. A broad spectrum of clinical illness can follow ingestion of *Salmonella typhi* with more severe forms being characterized by persisting high fever, abdominal discomfort, malaise and headache. Prior to the availability of antibiotics, the disease ran its course over several weeks, resulting in fatality [2].

The true burden of enteric fever in developing countries is difficult to estimate due to atypical clinical symptoms and subclinical infection similar to other febrile illnesses, making typhoid fever in endemic areas common, serious and increasingly difficult to treat as it remains unrecognized often [3,4]. In developing countries the improper medical facilities, ineffectiveness of diagnostics and lack of high standards in diagnostic laboratories had evolved typhoid as one of the complicated a health threats [5]. If the disease go undiagnosed in context to unavailability of definitive diagnostic procedures than it can lead toward the fatal complications like intestinal perforations [6]. Therefore, diagnosis based on clinical signs and symptoms alone is difficult. The emergence of multidrug-resistant *S. typhi* strains and development of the typhoid carrier state have further complicated the management of typhoid fever. Delay in diagnosis and initiation of antibiotic treatment can cause serious clinical complications and fatality. Thus, early and correct laboratory diagnosis of typhoid fever is critical to reduce the morbidity and mortality, as well as restrain transmission of the disease [7,8,9,17].

Current widely used methods for the diagnosis of individuals with enteric fever include bacterial culture, microscopy and serological assays, specifically the Widal test, which have been recently reviewed by Bhan et al. (2005), Bhutta

(2006), Kundu et al. (2006), Wain &Hosoglu (2008) and Parry et al. (2011) [10,11,12,13,14]. Molecular diagnostics of enteric fever, in particular nucleic acid amplification by Polymerase Chain Reaction (PCR), have been growing rapidly in last decade although they are confined within the research setting.

The typhoid bacilli was first observed by Eberth in 1880 in mesenteric nodes and spleen of fatal cases of typhoid fever and was isolated by Graffky, hence named as Eberth-Gaffky bacillus [15]. Historical records teach us a great deal about typhoid fever- the high disease burden and deadly consequences of the disease; and major routes of transmission. As early as 430 BC, a pernicious plague annihilated half the population of Athens, bringing to an end the "Golden Age of Athens," and is now believed to have been typhoid fever based on DNA examination of dental pulp. "Typhoid Mary" has become synonymous with the spread of the disease for more than a century, and William Budd, an English physician, demonstrated that typhoid fever could be transmitted through water sources in the 1870s. Thus, the person-to-person spread of the disease through food handling or via contaminated water has been documented for >100 years [16].

Classification

Ewing (1963) proposed a system of classification of the Enterobacteriaceae based on comparative studies of the biochemical reactions given by relatively large number of cultures of each genus and species within the family. The work of Fitts et al. (1983) confirms at the gene level that the salmonellae constitute a distinct group. Using one strain of S. typhimurium, 10 unique sequences of DNA were isolated that appeared to specifically hybridize with all the Salmonellae examined by the DNA-DNA hybridization technique [20].

The classification of Salmonella has been controversial for many years. Historically, the Salmonellae have received species rank based on their antigenic structure, more specifically on their Flagellar (H) and Somatic (O) antigen components. On this basis, Kelterborn gives a comprehensive account of the first isolation and geographical distribution of nearly 2,000 Salmonella serotypes. This compilation was based on the terminology introduced by Kauffmann and White. To date, there are more than 2,600 serotypes identified within the *Salmonella enterica* species [17,18,19]. A brief description of Salmonella species was given by Achtman M, et al. (2012) described as in (Figure 1).

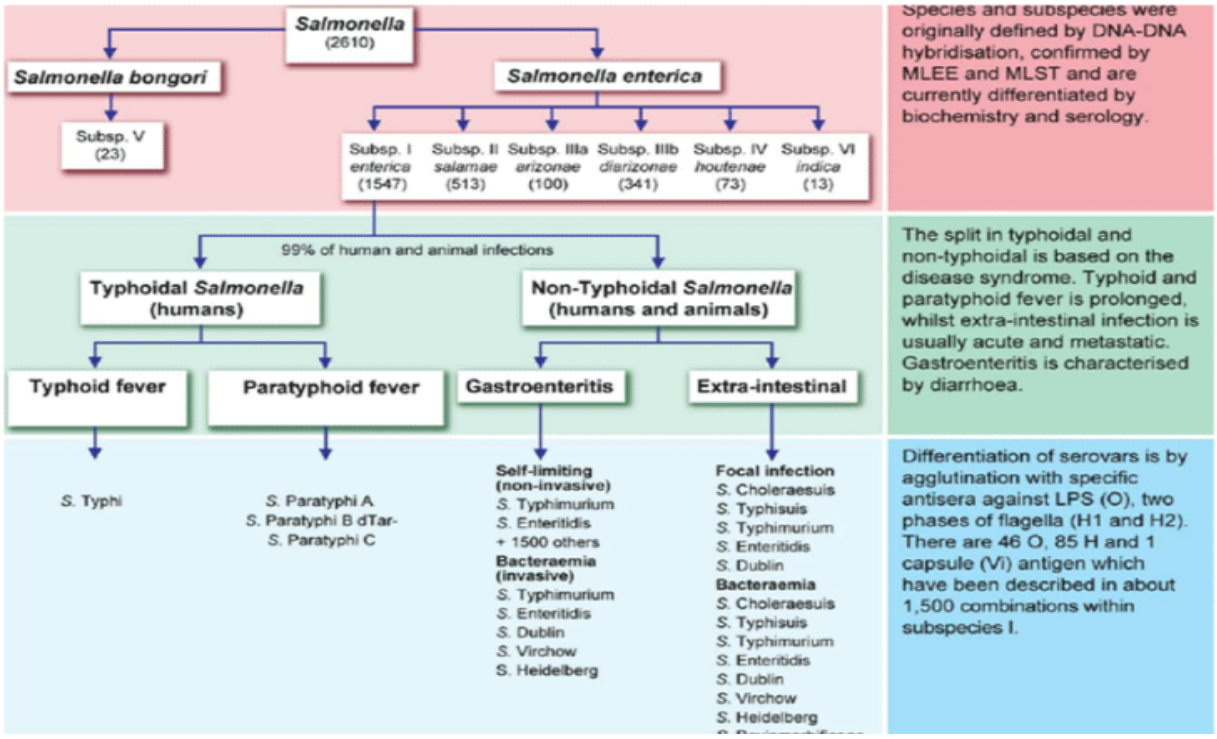

Figure 1: Details of classification and differentiating characteristics of Salmonella

Antigens

Salmonellae are Gram-negative, flagellated, *Facultative anaerobic bacilli* possessing three major antigens: H or flagellar antigen; O or somatic antigen and Vi antigen.

H antigen: Flagella confer motility to Salmonella and have been employed as one of the main antigenic determinants used for serotyping Salmonella isolates. The external body of the flagellum is mainly constituted by a homopolymer of flagellin, organized in a helicoidal distribution, presenting an exposed central

domain that is highly variable among the different serovars and conserved N and C-terminal regions that are responsible for the polymerization of flagellin to form the flagellum [21]. The variability of the central domain of flagellin is reflected in the high number of serovars described for the Salmonella genus.

The H or flagellar antigens, are heat-labile proteins located in the flagella. The H antigens in salmonellae have the almost unique character of biphasic variation. H antigens may occur in either or both of two forms, called phase 1 and phase 2 the organisms tend to change from one phase to the other. The H antigen of Salmonella comprise a large number of factors arranged in different combinations in the different serotypes [22]. Traditionally, Salmonella has been classified in serovars using defined procedures of agglutination, based on the different antigenic varieties of O and H surface antigens that correspond to LPS and flagellin molecules [23]. In most salmonellae the flagellar antigens exists in two alternative phases to identify a serotype completely it is necessary to determine the antigenic factors present in both phases.

Traditionally, serotyping was conducted using polyclonal antisera that were generated by inoculation with the desired strain and adsorbed with a set of related strains to increase the specificity of the resulting antiserum [24]. In the case of H antigen, preparation of specific antiserum was facilitated by the use of purified flagellin for immunization, although the presence of conserved regions in the molecule among different serovars originated possible cross-reactions depending on the procedure used for antiserum generation [25,26]. Upon the widespread use of techniques for Monoclonal Antibody (MAb) generation, several MAbs for strain serotyping have been described [27,28]. The availability of strain-specific MAbs for serotyping provides many advantages, such as using a molecularly defined homogeneous reagent that can be produced in high scale and basically without restrictions of quantity [29].

O antigen: Lipo Polysaccharide (LPS) is composed of a lipid a tail, which anchors the LPS into the membrane; a core oligosaccharide; and an O-antigen side chain. The O antigen is not a single factor but a mosaic of two or more antigenic factors. Salmonella is classified into a number of groups based on the presence of characteristic O antigen on the bacterial surface [15]. The O polysaccharide have a core structure that is common to all enterobacteria and side chains of sugars attached to the core determine O specificity [22]. The O-antigen side chain of the lipo polysaccharide is an immunodominant antigen, can define host-pathogen interactions [30].

The O antigen is a heat-stable polysaccharide part of the cell wall LPS. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface [31]. The surface-exposed O-antigen side chain protects the bacterial cell from the actions of the innate immune system [32]. The O-antigen is immunogenic and may be a functional target for novel vaccines [33,34]. The *S. enterica* subspecies is comprised of over 2,600 serovars, which are based on differences in the antigenic properties of the O- and H (flagellar)-antigens and form the basis of the Kauffman-White serotyping system [18,30].

Salmonellae may be classified into 'chemotypes' each of which contains organisms with the same sugars in their side chains. Chemotypes may include salmonellae with different O antigens and are serologically distinct because of difference in the sequence of sugars in the side chains, particularly at the distal end [22]. During natural infection, antibodies are raised against LPS and the detection of *S. typhi* O-antigen antibodies forms the basis of the diagnostic Widal test for typhoid [36]. The composition of the O-antigen can be modified by the activity of Glycosyltransferase (gtr) operons acquired by horizontal gene transfer [30].

Vi antigen: The Vi capsular polysaccharide (Vi) is a component of several members of the family Enterobacteriaceae, including *Salmonella typhi, Salmonella paratyphi C* the Vi polysaccharide acts as a virulence factor by inhibiting phagocytosis, resisting compliment activation and bacterial lysis by alternative pathway and peroxidase mediated killing [18,30,37,38,15].

Vi antigen is a superficial antigen overlying the O antigen; surface polysaccharides that inhibit the agglutinability of the organisms by homologous O antisera [22]. Bacterial surface antigens play a key role in determining the interaction between the pathogen and the host. Studies of the Vi-Ag capsule have greatly increased our understanding of the pathogenic mechanisms of *S. typhi* and continue to yield promising options for vaccine candidates. The Vi-Ag capsule is important for systemic dissemination of *S. typhi* and although the polysaccharide is absent from the surface of *Non-typhoidal Salmonella,* NTS are known to result in extra intestinal infection in up to 5% of cases [39,40,41].

Transmittance and Host Range

The epidemiology of typhoid fever are human host–restricted and primarily involves person-to-person spread because these organisms lack a significant animal reservoir. Contamination with human feces is the major mode of spread, and the usual vehicle is contaminated water, food, raw fruits, vegetables, ice-creams, juices and contact with infected patients or carrier of the diseases [42]. It has been observed that in developed countries the source of outbreak is food while in developing countries the disease spread mostly by contaminated water [43,44]. Travelers, children, the elderly and immune-compromised individuals are especially at risk. The children aging 2-10 years and young population up to 25 years of age irrespective of their sexes suffer from enteric fever and are admitted to the hospitals in the endemic regions [45,86].

Symptoms and Signs

The genus Salmonella consist of bacilli that are parasites in the intestine of large number of vertebrae species and infects human beings leading to enteric fever, septicemia, with or without focal suppuration and the carrier state [17]. Recent human challenge studies have also demonstrated that a proportion of patients develop a subclinical or asymptomatic

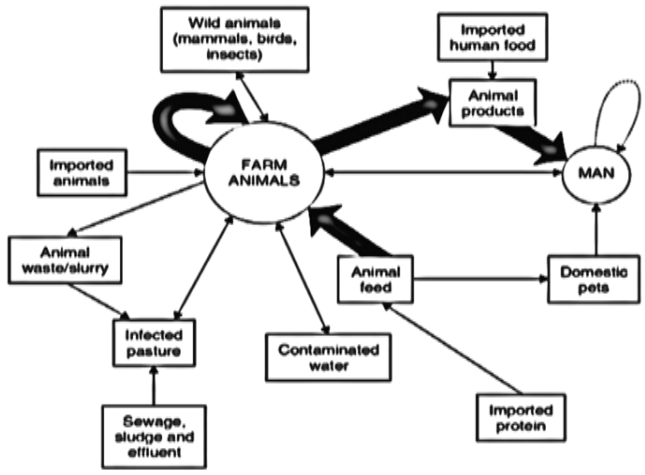

Figure 2: Transmittance and host range

bacteremia and that fecal shedding can occur in the period before symptom development, during primary infection [57]. As symptomatic disease develops, the predominant symptom is the fever [46,48,58]. The temperature rises gradually during the first week of the illness and reaches a high range of 39 to 40°C the following week. There is little diurnal variation, although the pattern may be modified by anti-pyretic medications. Patients can have influenza like symptoms, a dull frontal headache, malaise, anorexia, a dry cough, sore throat, and occasionally epistaxis. Constipation is a frequent early symptom although many patients will experience diarrhoea at some point. Patients may have abdominal pain that is diffused and poorly localized. Nausea is common, and vomiting occurs in more severe cases. It is unusual for a patient hospitalized with typhoid to have no abdominal symptoms and normal bowel movements [59].

In case of milder disease, self-medication, or treatment in health stations, clinics, or as hospital outpatients patients do not require admission to hospital [49–51]. These improperly managed cases may be of nonspecific illness that is not recognized clinically as enteric fever, especially among children under 5 years of age [52–54]. After ingestion of *Salmonella serovar typhi* or *Paratyphi A*, an asymptomatic period follows that usually lasts 1-2 weeks. A higher infecting dose leads to a higher attack rate and a shorter interval to bacteremia but has no influence on the time to symptom development or disease severity [55,56].

Clinical syndromes caused by Salmonella infection in humans are broadly divided into two groups. The first is systemic infection is characterized by septicemia and ultimate localization in extra-intestinal sites [101]. The gastrointestinal form is often referred to as the 'food poisoning syndrome,' although this is a misnomer, as the disease is an infection rather than an intoxication. The systemic disease is referred to as enteric fever, it is transmitted by contaminated water or food, and is caused mainly by *Salmonella enterica serovar typhi* (typhoid fever) or *Salmonella enterica serovar Paratyphi* A, B or C (paratyphoid fever) [31,60,97].

Complications

Typhoid has a high prevalence in tropical countries. The typical symptoms may not be seen in all patients and the disease may have unusual manifestations. Typhoid fever may either be a primary or secondary event leading to acute acalculous cholecystitis. Routes of gallbladder infection by typhoid bacilli may be either through blood stream, biliary system or contiguous infected organs or lymphatics from gastrointestinal tract [61,62].

The disease is characterized by prolonged fever, and constitutional symptoms including headache, anorexia and abdominal pain [58]. The systemic involvement in typhoid fever can result in extra-intestinal complications such as encephalopathy, meningitis, hepatitis, myocarditis and pneumonia, while the most common gastro-intestinal complication is haemorrhage. Neurological complications are not uncommon. In children, known and reported neurological complications are encephalopathy, meningism, spastic paralysis-cerebral origin, convulsions, meningitis, parkinsonian syndrome, sensory motor neuropathy, cerebellar involvement, and schizophrenic psychosis [64,65].

Surgical complications of typhoid usually involve the small gut rather than the gall bladder and *Salmonella typhi* is more common than *S. paratyphi.* In the gut, it commonly causes intestinal perforation, resulting in high morbidity and mortality. It rarely causes acute cholecystitis, gangrene or perforation of the gall bladder. When these two complications coexist mortality rate is increased [66]. Intestinal perforation is a potentially fatal complication of typhoid fever secondary to the inflammation and necrosis of Peyer's patches when not treated early and appropriately. Generally, perforation is a late complication occurring in the third week of illness, though it is reported earlier in second week in developing countries for reasons that are not completely understood [64,67,68].

Pathogenesis

Salmonella pathogenicity is mediated mainly by horizontally transferred chromosomal regions, encoding sets of virulence factors enabling the pathogen to successfully infect and colonize its host [69].

Specialized epithelial cells overlaying Peyer's patches known as M cells, are probably the site of internalization of Salmonella and its transport to underlying lymphoid tissue. During the bacterimic phase, the organism disseminated widely and the most common sites of secondary infection are the liver, spleen, bone marrow, gall-bladder and Peyer's patches of the terminal ileum [58]. After penetration, the invading microorganisms translocate to the intestinal lymphoid follicles and the drained mesenteric lymph nodes and some can pass onto the reticulo-endothelial cells of the liver and spleen [71,72]. The incubation period is usually 7 to 14 days. In the bacteremic phase, the organism is widely disseminated. The most common sites of secondary infection are the liver, spleen, bone marrow, gall bladder and Peyer's patches of the terminal ileum. Gall bladder invasion occurs directly from blood or by retrograde spread from

the bile. Typhoid induces local and systemic humoral and cellular immune responses, but these do not confer complete protection against reinfection and relapse [73,74].

Multidrug-Resistance

Multidrug-Resistant (MDR) *Salmonella typhi* strains are spreading in various countries of world. Studies have been conducted to define the antibiogram pattern of *Salmonella typhi* and *S. paratyphi* A and these shows that the multiple antibiotic resistance is emerging in India [75,76,77]. Multidrug resistant typhoid and paratyphoid infections are more severe with higher rates of toxicity, complication and mortality than infections with sensitive strains. This may be related to the increased virulence of multidrug resistant Salmonella as well as a higher number of circulating bacteria [78].

The emergence of drug resistant typhoid has been another worrying development. After sporadic outbreaks of chloramphenicol resistant typhoid between 1970 and 1985, many strains of *S typhi* developed plasmid mediated multidrug resistance to the three primary antimicrobials used (ampicillin, chloramphenicol, and Co-trimoxazole). This was countered by the advent of oral quinolones, but chromosomally acquired quinolone resistance in *S typhi* and *S paratyphi* has been recently described in various parts of Asia, possibly related to the widespread and indiscriminate use of quinolones.

Since the late 1980s, Multidrug-Resistant (MDR) strains of *S. typhi* that exhibit resistance to chloramphenicol, ampicillin and trimethoprim have increased and spread in Southeast Asia, Central Asia, South America and Africa [79,80]. In recent decades, ~10,000–20,000 typhoid cases have been reported annually in China, and outbreaks are not uncommon. The emergence of MDR *S. typhi* results in the excessive use of ciprofloxacin for typhoid fever treatment. In the 1980s, ciprofloxacin became the first-line drug to treat *S. typhi* infections after most of the conventional drugs became ineffective. However, resistance to ciprofloxacin was observed in the early 1990s, and treatment failure with this antibiotic was reported by the late 1990s [81].

The emergence and spread of Salmonella isolates presenting resistance to several antibiotics is matter of concern because these medicines are crucial to the successful treatment of invasive infections. Since resistance to older antibiotics (e.g. ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole) has been increasing for many years, recommended treatment options for salmonellosis included fluoroquinolones (ciprofloxacin) and extended-spectrum cephalosporins [82,83]. However, resistance to those 'critically important antibiotics for human health' is emerging, leading to increased severity, morbidity and mortality of diseases and the need for the use of last-line antimicrobials in therapy [84].

For several decades, the contribution of the food-animal as a reservoir of antimicrobial resistance with impacts on human health has been controversial, but accumulating evidence linking particularly poultry production with human disease have been reported, namely involving *Non-typhoidal Salmonella.* In middle-income countries such as in the Asiatic continent, resistance to ciprofloxacin and extended-spectrum cephalosporins is currently a growing problem, with poultry products playing a potential role in its emergence [83,84].

Non-typhoidal Salmonellae

Salmonella enterica serovars typhi, Paratyphi A, Paratyphi B, and *Paratyphi C* may be referred to collectively as typhoidal Salmonella, whereas other serovars are grouped as *Non-typhoidal Salmonella* (NTS). The Salmonella that induce human diseases are divided into typhoidal serotypes (*S. Typhi* and *S. Paratyphi* A and B) and thousands of non-typhoidal serotypes [85]. Salmonellosis is caused by *Non-typhoidal Salmonella enterica* serotypes and is typically characterized by a self-limiting gastroenteritis syndrome, diarrhoea, fever and abdominal pain, with an incubation period between 4 and 72 hour and mortality being rare [84]. NTS strains may be host generalists, infecting or colonizing a broad range of vertebrate animals, or may be adapted or restricted to particular nonhuman animal species [86].

Transmission of the infection is through the fecal-oral route via contaminated water and foods. This is the reason of endemicity in underdeveloped and developing countries where the sewage systems can mix with drinking water as the infrastructure are inadequate [87,88]. The Salmonella infection most commonly presents itself as gastroenteritis, also called as minor salmonellosis, and usually lasts 3-7 days. Temporary bacteraemia can be seen in a small number of patients (1-4%) and some of the patients need to be hospitalized due to dehydration. A mild increase in C-Reactive Protein (CRP), the Erythrocyte Sedimentation Rate (ESR) and leukocytosis can be seen [89].

Non-typhoidal Salmonella infection causes 155.000 deaths with 93.8 million diarrhoea cases per year [6]. Among the gastroenteritis patients in various parts of the world, the detection of Salmonella is between 2.66 and 15.2% [90]. The rate of Salmonella detection was 5.2%. In other publications from our country, this ratio is between 1 and 2.5% [91]. Different rates of detection are not only dependent on local epidemiological dynamics but may also relate to the analytical and diagnostic performances of microbiological methods used for diagnosis [89]. Recent estimates of typhoidal Salmonella incidence have varied substantially and NTS estimates are sparse in large part due to poor access to reliable diagnostics, particularly in low resource outpatient settings where patients with these illnesses typically present for medical care [92,93,94,95].

Invasive Salmonellosis is perhaps the most important infectious disease cluster for which rapid and reliable diagnostics do not exist. Essentially all enteric fever diagnosis begins with evaluation of clinical signs and symptoms. For perhaps the majority of patients with suspected enteric fever worldwide, who live in settings where diagnostic microbiology is unavailable [95].

Burden of Disease

Typhoid fever is an exclusively human enterically transmitted systemic disease caused by infection with the bacterium *Salmonella enterica serovar typhi.* Worldwide, typhoid

fever causes 269,000 deaths from 26.9 million new cases each year [94]. In relation to typhoid fever, it has been widely assumed that the incidence and mortality is low; that disease principally occurs in older children and adults; that multidrug resistance, although a pressure on resources, has little effect on epidemiology; and that *Salmonella typhi* within households is due to a common source rather than multiple sources [44].

Typhoid and *S. paratyphoid* fevers were included in the Global Burden of Disease 2010 project, when they were together estimated to account for 12.2 million disability-adjusted life years and 190,200 deaths [86,96]. An estimate found that 22 million new typhoid cases occur each year in the world with some 200,000 of these resulting in death [97]. Although largely controlled in Europe and North America, typhoid remains endemic in many parts of the world. Typhoid fever incidence varies substantially high areas of endemicity include South-central Asia, Southeast Asia, and Southern Africa [98,99].

The highest number of cases (100 per 100,000 persons/ year) and consequent fatalities are believed to occur in South Central and Southeast Asia. Typhoid burden suggests that most of sub-Saharan Africa now experiences typhoid fever incidence >100 per 100 000 persons per year, with 33 490 deaths, 26.0% of global typhoid deaths, occurring in Africa [44,97]. On the Indian subcontinent, Pakistan has the highest incidence (451.7 per 100,000 persons/year) of typhoid fever followed by India (214.2 per 100,000 persons/year). In the United States, 200–300 new cases are reported annually, most of which occur in travelers returning from endemic countries [76,97,100].

Prevention and Control

Life Style and Community

The infection by *Salmonella typhi* can be prevented by hygienic life style, clean water and fresh food supplies. This simple method is nearly impossible to achieve in Asian countries due to unavailability of clean water and hygienic conditions. Typhoid has been controlled all over the world with the use of various vaccines and mass vaccination programs in various countries.

As humans are the only reservoir of this faeco-orally transmitted disease, preventive measures include improvement of water supply and sanitation facilities. However, instituting these measures requires a huge investment, making it an almost unachievable task, especially in resource-poor countries where they are needed most. For comprehensive control measures, cases need to be diagnosed early followed by provision of prompt and appropriate treatment. Carriers need to be identified efficiently and early treatment instituted [101].

Vaccines

The first vaccines against typhoid fever consisting of heat-inactivated typhoid bacilli preserved in phenol administered parenterally, were developed in the late 19th century. Experiences with implementation of typhoid vaccines in the British and US military in the early 20th century and subsequent large-scale controlled field trials sponsored by the World Health Organization documented that the inactivated whole cell vaccines were efficacious but were highly reactogenic. Whole-cell vaccines against Salmonella enterica serovars paratyphi A and B were also developed in the early 20th century and used by the U.S. military as a trivalent vaccine against enteric fever [102,103]. However, these whole-cell vaccines lost favor due to their propensity to produce high fever, severe headache and malaise and gave way to the development of better tolerated Salmonella vaccines using other approaches such as parenteral polysaccharide and polysaccharide-protein conjugate vaccines and live attenuated oral vaccines [104].

Three types of Salmonella vaccines are licensed: the oral live attenuated *Salmonella typhi* Ty21a vaccine and the parenteral Vi capsular polysaccharide antigen, either unconjugated or conjugated to tetanus toxin [105–106]. Vi capsular Polysaccharide (Vi-PS) vaccines confer protection approximately 7 days post immunization. As is typical for polysaccharide vaccines, they do not induce protective immune responses in children <2 years of age [107,108].

Live Attenuated Vaccines

The live oral vaccine is a stable mutant of *S. typhi* stain Ty21a, lacking the enzyme UDP-galactose-4-epimease. On ingestion, it initiates the infection but 'self-destructs' after four or five cell divisions, and therefore cannot induce any illness. The vaccine is developed in two formulations: enteric-coated capsules and a liquid presentation. The commercially available enteric-coated capsules are intended for use in children >5 years of age and adults [15,107]. The vaccine is an enteric coated capsule containing 109 viable lyophilized mutant bacilli. The course consists of one capsule orally, taken an hour before food, with the glass of water or milk, on day 1, 3 and 5. No antibiotics should be taken during this period [15].

There are several advantages of live oral attenuated vaccines over other vaccine formulations: 1) they can induce local immune responses at mucosal surfaces; 2) they are economical to produce; 3) they induce Salmonella-specific B and T cell immunity; 4) they are practical to administer to a large population [109,110]. However, there are several limitations to live attenuated vaccines. First, one needs to balance immunity and reactogenicity, particularly if the vaccine is to be used as a live vaccine vector. The vaccine may also need to be formulated differently for infants. For example, Ty21a at times has been available in both a sachet formulation for use in young children as well as enteric-coated capsules for use in older children and adults [106–111]. Finally, safety of live attenuated vaccines needs to be determined in immuno compromised subjects and also the very young prior to wide spread use.

Vi-Polysaccharide Vaccine

Vi vaccine is based on the *Salmonella serovar typhi* Vi capsular polysaccharide antigen. It is licensed for children aged under 2 years. The effectiveness of the parenteral Vi vaccine has recently been confirmed in young children [112,86]. There are mainly two types of Vi polysaccharide vaccine: unconjugated and

conjugated.

Injectable Vi Polysaccharide (Unconjugated)

Vaccine is a subunit vaccine, first licensed in the United States in 1994. It contains purified Vi capsular polysaccharide from the Ty2 *S. typhi* strain. It is injected intramuscularly. It is given as a single dose to children older than 2 years with subsequent shots at every 3 year interval or at least one booster dose after 3 years.

Vi Polysaccharide Conjugate

Vaccine is a Vi capsular polysaccharide linked to a conjugate. Vi conjugate vaccine provides longer lasting immunity as it has the ability to stimulate specialized cells in the human body called T cells, which the Vi polysaccharide alone cannot do. Vi conjugate vaccines are the only vaccines advisable for kids below 2 years of age [114]. Subunit vaccines can be given to people with weakened immune systems. These vaccines appear to give long-lived immunity. Since only parts of the virus are used for these vaccines, the risks of reactions are very low. Several doses must be given for proper life-long immunity.

Salmonella Paratyphi A Vaccines

Currently the emergence of *Salmonella paratyphi A* is getting prevalent, so the vaccine for *Salmonella paratyphi A* had become a matter of concern as there are no currently licensed vaccine against *S. paratyphi A.* There for new vaccination strategies need to be followed to develop novel vaccines for paratyphoid to overcome the burden of enteric fever caused by the Salmonella paratyphi A infections.

Many attempts have been made by various workers to develop a suitable vaccine against *S. paratyphi A.* Two recombinant antigen SpaO and unique flagellin subunit H1a of *Salmonella paratyphi A* were developed and were used to test the immunogenicity in mice [115]. Studies of different Salmonella paratyphi A strains has been to develop a live attenuated paratyphoid vaccine [116]. Outer membrane proteins of S. paratyphi A CMCC50973 strain induced imuno-protection in a challenge experiment on vaccinated mice [117]. Role of flagella of *S. paratyphi A* as a potential protective antigen was studied as a rationally attenuated S. paratyphi A live vaccine which had induced the immune responses for the flagellar protein [118]. The immunogenic properties of *Salmonella typhi* capsular polysaccharide- diphtheria toxoid conjugates has been checked in mice [119]. A *Salmonella paratyphi A* vaccine has been developed by the NIH, Bethesda and is being studied in field trials in Vietnam. It consists of the detoxified O-specific polysaccharides of *S. paratyphi A* that have been conjugated with tetanus toxoid. It has been shown to be safe and able to elicit IgG antibodies with bactericidal activity in the serum of adults, teenagers and 2 to 4 year old children [120].

Diagnosis

Widal Test

The first typhoid diagnostic, the Widal test, was developed in 1896; it is a visual test that monitors agglutinating antibodies that react with S. typhi. In 1896, Widal reported that sera from patients with clinical typhoid 'clumped' the bacteria isolated from the host and this agglutination method formed the basis of serodiagnostic tests for typhoid for over 100 years [121]. Traditionally, the Widal assay involved an agglutination assay using formalin-fixed bacteria expressing the LPS and flagellar antigens, with antibodies to the Vi capsule being detected using a strain of S. typhi expressing a Vi capsule but lacking LPS and flagella antigens. The Widal agglutination assays were refined and standardized by many researchers. Over the following years, the assay was modified in both the test and the interpretation of the results [122].

Bacterial Culture

Salmonella are aerobic and facultative anaerobic, growing rapidly on simple media over a range of pH 6-8 and optimum temperature of 37^0 C. It may be cultured on a variety of solid media; on selective media for primary isolation (Blood Agar), Selective or differential agar (e.g.: MacConkey Agar, Hektoen Enteric Agar) and Enrichment broths (e.g.: Selenite broth). S. typhi produce non-hemolytic smooth white colonies on blood agar, non-lactose fermenting smooth colonies i.e. pale colonies on MacConkey agar and on Hektoen enteric agar, salmonellae produce transparent green colonies with black centers [16]. Salmonella is characterized by their ability to metabolize citrate as a sole carbon source and lysine as a nitrogen source, as well as their ability to produce hydrogen sulfide [1]. Salmonella ferment glucose, mannitol and maltose, forming acid and gas. They do not ferment lactose, sucrose and salicin. Indole is not produced, are VP negative and methyl red positive [15].

Bacterial culture is clearly the method of choice in situations where laboratory facilities to perform the assay and the required typing procedure are available. The culture of S. typhi can be done from many body fluids such as blood, bone marrow, stool, rose spot extracts, duodenal aspirates and urine, while the blood culture remains the mainstay of definitive diagnosis [123,124].

Blood Culture: In developing countries like India sensitivity of blood culture is lowered due to irrational use of antibiotics. Blood culture is generally considered the standard method for diagnosis of typhoid bacteremia but has been shown to detect only 40 to 70% of typhoid patients [125]. Culturing is an accurate method for the diagnosis of typhoid fever for blood samples drawn early in the disease. More than 70% of the cases will be confirmed when multiple blood samples are tested [126,127]. The detection rate of the culture was 65.9% for single blood samples drawn an average of six days after the onset of fever [128,129]. Tryptone soy broths are among the most commonly used blood culture media today, with automated systems used in settings with sufficient resources. The majority of positive cultures are evident within 48 hours, and nearly all are positive by five days [130].

Febrile patients often take antibiotics; self-administered, prescribed or un-prescribed, which reduces the possibility of bacterial growth because antibiotics may inhibit

the growth of *S. typhi.* In a quantitative study of bacteremia in typhoid fever patients, 50% of patients identified by positive blood culture had less than 1 CFU/ml of blood, illustrating the need for highly sensitive methods of detection [131]. However in untreated patients with enteric fever, the blood culture is positive in 80% of patients or more. The optimum period for detecting organisms circulating in the bloodstream is considered to be in the first or second week of the illness, although cultures can still remain positive in the third week in the absence of antimicrobial exposure [132,133]. Quantitative bacteriology studies have shown declining counts with an increasing duration of disease [131]. Specimen collection, storage and transportation condition is likely to affect blood culture yield besides the culture media used.

Bone Marrow Culture: Bone marrow cultures have the highest sensitivity (>80%) and are relatively unaffected by antibiotics [131,135]. The increased sensitivity of bone marrow culture compared with blood culture relates to the higher bacterial concentration in bone marrow. Bone marrow culture is more frequently positive in patients with severe and complicated disease [131,136].

Fecal Culture: *Salmonella enterica* may be isolated from feces in up to 30% of patients with typhoid fever and in 1% of urine samples, with the number of organisms recoverable from feces increasing throughout an untreated illness. The sensitivity of fecal culture increases from about 10% in a single sample to about 30% by testing multiple samples. Sensitivity is also improved by using whole feces rather than rectal swabs and by using a Selenite F enrichment step and selective media. Culture of bile obtained from an overnight duodenal string capsule provides a sensitivity similar to that for blood culture and offers an additional means to isolate typhoidal Salmonella from patients or carriers [131].

Disadvantages of culture are that it can be done only by few specialized laboratories, it requires a relatively large volume of blood collected by venipuncture, and the result is obtained only after 2–3 days. A further disadvantage is that multi blood cultures must be performed to ensure a high sensitivity and to exclude contamination. However, the use of antibiotics, low amount of bacteria, amount of blood used to inoculate the culture may affect the detection rate of culture. Culture is expensive method which takes 2–3 days for results, requires lab facilities and trained staff to perform the culture. All these issues cumulatively contributed to the relatively low sensitivity of the culture procedure [128,129].

Molecular Assays

Rapid molecular detection methods, such as nucleic acid-based amplification, such as PCR assay, is critically needed to help diagnose this contagious disease. Development of this test requires diagnostic markers that are sensitive and specific [17]. DNA-based detection methods, such as Polymerase Chain Reaction (PCR), have proven to be sensitive, specific, and rapid compared to conventional culture-based methods for the diagnosis of many infectious diseases.

Nucleic acid amplification tests, including conventional PCR and real-time PCR, have been developed for the detection of both *Salmonella serovar S. typhi* and *Paratyphi A*, mainly in blood. Nucleic acid amplification methods have the potential to amplify small numbers of organisms and non culturable bacteria, as well as dead organisms. Targets for *Salmonella serovar typhi* PCR-based assays have included the Hd flagellin gene fliC-d, the Vi capsular gene viaB, the Tyvelose epimerase gene (tyv) (previously rfbE), the paratose synthase gene (prt) (previously rfbS), groEL, the 16sRNA gene, hilA (a regulatory gene in Salmonella pathogenicity island 1 [SPI-1], the gene encoding the 50-kDa outer membrane protein ST50 O antigen somatic genes (tyv and prt), and the hypothetical protein gene rat A [63,70,11335,134,137,138].

However, these genes cannot stand alone as single *S .typhi* specific diagnostic marker since they are not specific to *S. typh*i and are also found in other Salmonella serotypes. Thus, these markers provide provisional rather than differential diagnosis of typhoid fever. For example, the fliC-d gene of *S. typhi* shares the same nucleic acid sequence as *S. muenchen* the protease gene is present in *S. typhi, S. Paratyphi A*, and *S. enteritidis;* and the via B gene is found not only in S. typhi but also in *S. Dublin,* a few strains of *S. Paratyphi C* and *Citrobacter freundii* [139,144,148].

Due to the lack of specificity of these target genes, a combination of different pairs of primers using multiplex PCR or nested PCR are needed to increase the sensitivity and specificity of the PCR diagnostic test. Nested primers have been used in some studies to improve sensitivity, although this may lead to unspecific amplification and contamination. This, however, will increase the cost, time, and complexity of the laboratory diagnosis [17].

Diagnostic markers which can detect pathogens at single gene target resolution could lead to a simpler, cost-effective, and more functional DNA-based detection method since less primers are needed for target detection. Many approaches, such as subtractive hybridization, next generation sequencing, and microarray techniques, have been used to identify genes that are specific or unique to a pathogen [17]. A nested polymerase chain reaction using H1-d primers has been used to amplify specific genes of S. typhi in the blood of patients and is a promising means of making a rapid diagnosis [149].

Five *S. typhi*-specific genes, namely, STY0307, STY0322, STY0326, STY2020, and STY2021, were found to be highly conserved among *S. typhi* strains and showed sequence homology to proteins of known function using protein BLAST (BLASTp) programs. Genes STY0307, STY0322 and STY0326 encode for hypothetical proteins, while genes STY2020 and STY2021 encode for putative bacteriophage proteins. The genes STY0307, STY0322 and STY0326 are located in the Salmonella Pathogenicity Island 6(SPI-6) [17].

Moreover the gene STY0201 has been also used as a PCR target, and the PCR assays that were developed based on this gene were reported to be 100% sensitivity and specificity [160]. However, this study found that this gene was only 97.2% specific and cross-reacted with *S Oslo* and *S. Kissi.* The incorrect bioinformatic prediction of the specificity of gene STY0201 may

be due to the incomplete genome sequence available for the 2 bacteria in the NCBI database that limit the matching accuracy of the BLASTn search [161].

Rapid Serologic Tests

The need for a rapid, inexpensive and user friendly laboratory test for early and accurate diagnosis of patients with typhoid fever has prompted the exploration of a variety of serologic and antigen detection methods, including counter immuno-electrophoresis, Enzyme-Linked Immunosorbent Assay (ELISA), dot immunoassay, hemagglutination and coagglutination. However, these assays are not very easy to perform, not rapid, require special equipment or skills, or depend on electricity and on refrigeration for storage of components. None of these assays has yet reached widespread use.

Countercurrent Immuno electophoresistest is based on the appearance of the precipitation band of antigen-antibody complexes that form on electrophoresis. The sensitivity is similar to that of the Widal test and the procedure may be quicker if tests are batched (about one hour for a gel), but bands are often difficult to see, the cost is higher than that of the Widal [140].

Mochammad et al, 2002 developed a simple dipstick assay for the detection of *Salmonella typhi*-Specific IgM antibodies and the evolution of the immune response in typhoid patients [141]. The study indicated a sensitivity of 65.3% for the dipstick assay for samples collected at the time of hospital admission from the *S. typhi* and *S. Paratyphi* culture-positive patients.

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen of interest can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. Antigens from the sample are attached to a surface. A further specific antibody is applied over the surface. So it can bind to the antigen. This antibody is linked to an enzyme. In the final step a substance containing the enzyme substrate is added. The subsequent reaction can produce a detectable signal, most commonly a color change in the substrate [142,143,144].

New Diagnostic Approaches

Newer diagnostic tests have been developed—such as the Typhidot or Tubex which directly detect IgM antibodies against a host of specific *S. typhi* antigens—but these have not proved to be sufficiently robust in large scale evaluations in community settings [142,143].

Other than culture and tube agglutination test, a dot blot assay detecting IgM and IgG antibodies against *Salmonella enterica serovar typhi* can help in early diagnosis using a composite reference [4]. Newer diagnostic tests have been developed—such as the Typhidot or Tubex which directly detect IgM antibodies against a host of specific S. typhi antigens—but these have not proved to be sufficiently robust in large scale evaluations in community settings.

Attempts have been made to develop simple, accurate, rapid and inexpensive methods to detect salmonella infection. Among them, the most favorable method is enzyme immunoassay because it is sensitive and rapid, permits multi sample analysis at one time, and requires only a minimum of equipment. Castro NC and Fmrex PA 1998 had worked of dot-blot ELISA for typhoid fever detection [145]. They used the crude LPS and the flagellar antigen as blotting antigens on Nitrocellulose membrane. The serum samples were treated with goat antihuman anti-IgG or anti-IgM and diaminobenzidine was used as the substrate. The membrane after getting dry gave the brown colour visualization in the positive samples and pink colour in the negative samples. The dot-blot test detecting IgM against flagellar antigen had a lower sensitivity (12.1%) than the Widal test but a higher specificity (97%).

The dot-blot sensitivity and specificity obtained by Choo et al, 1994 using a 50-kDa outer-membrane protein of S. typhi to detect IgG antibodies were 95% and 75% respectively [146]. In a similar study by Chaicumpa et al, 1995 modified a monoclonal antibody based dot blot assay to be used for the detection of Salmonella in food items [147].

Antibody Detection

Culturing the bacteria from body fluids is the definitive test for the diagnosis of typhoid fever although inconclusive serological methods such as Widal test are commonly employed in many health care settings [65].

The Widal Test: Measures agglutinating antibodies against LPS (O) and flagellar (H) antigens of Salmonella serovar typhi in the sera of individuals with suspected enteric fever [107]. Although usually discouraged due to inaccuracy, it is simple and inexpensive to perform and is still widely used in some countries [150]. The performance of the method has been hampered by a lack of standardization of reagents and inappropriate result interpretation [151]. The Widal test ideally requires both acute- and convalescent-phase serum samples taken approximately 10 days apart; a positive result is determined by a 4-fold increase in antibody titer [60].

Tubex: TF is based on an inhibition reaction between patient antibodies (IgM) and monoclonal antibodies included in the test that bind to a *Salmonella typhi* specific O9 lipopolysaccharide. A macroscopically visible de-colorization of patient serum in test reagent solution through magnetic particle separation indicates a positive result [163]. The test is a semi-quantitative competitive agglutination test for the detection of anti-O9 antibodies [164].

In contrast the typhidot is based on a qualitative dot-blot enzyme-linked immunosorbent assay that separately detects the presence of IgM and IgG in patient sera against a *Salmonella Typhi* specific 50 kD outer membrane protein. This test is further modified by using the cassettes of combined antigens or by using it in a dipstick assay [165].

Enzyme-Linked Immunosorbent Assays: ELISA have been considered an alternative approach for the diagnosis of typhoid fever. The IgM response to successfully treated bacterial infections generally persists for only a few weeks or months. Demonstration

of IgM antibodies to Salmonella antigen might therefore be of more diagnostic significance in an endemic population than detection of IgG. However, there are theoretical limitations to the indirect ELISA for IgM. If the concentration of specific IgG in a sample is substantially greater than the concentration of IgM, it can produce false negative results by competing for the antigen determinants on the plate. On the other hand, an IgM-class rheumatoid factor that may be present in the sample can react with antigen-IgG complexes and produce a false-positive result [166].

The ELISAs are quantitative, samples do not need pre-assay handling, sample purity is not required and the overall running costs are lower. Further more, the results of an ELISA can also be easily interpreted using simple relation to a standard curve. ELISAs can confirm infection in all phases of the disease and will additionally identify carriage of the bacterium in asymptomatic humans, particularly relevant for research purposes.

A summary of various antibody detection tests available in global market are summarized in (Table 01).

Table 1: Tabular comparison of various antibody detection tests available in market.

S. No.	Test Name	Principle	Features
1.	Multi-Test-Dip-Sticks	Dipstick detecting anti-LPS IgG and IgM	Sensitivity, 89%; specificity, 53%
2.	Tubex TF	Detects antibody against *Salmonella typhi* LPS with an inhibition assay format and a visual result readout	Sensitivity, 56–100%; specificity,58–100%
3.	Typhidot	Measures IgM and IgG antibodies against a 50-kDa outer membrane protein of *Salmonella typhi* in an immunodot test format.	Sensitivity, 67–98%; specificity,58–100%
4.	Typhidot M	Measures IgM antibodies, after removal of IgG antibodies, against a 50-kDa outer membrane protein of *Salmonella typhi* in a dot blot format.	Sensitivity, 47–98%; specificity,65–93%
5.	Typhi Rapid IgM and IgG IgM (Combo)	Measures IgM antibodies, after removal of IgG antibodies, against a 50-kDa outer membrane protein of *Salmonella typhi* in an ICT LFA a cassette format.	Sensitivity, 89–100%; specificity,85-89%
6.	Widal test	Measures agglutinating antibodies against O and H antigens of *Salmonella typhi* and *Salmonella Paratyphi A*; uses a tube or slide format.	Very variable sensitivity and specificity; lack of standardized reagents

Currently the best available test for the diagnosis is typhidot which is based on the detection of the IgM antibodies detection in the serum of the patient. It is easy to perform and provides the clear results for the recent infection with the bacteria moreover by IgG detection the carrier stage of the bacteria can also be described. This test had been validated on clinical parameters in various developing countries including India.

Blood serum is a better sample for the diagnostic purposes as it helps in the qualitative as well as quantitative estimation of the various antibodies developed due to the infection.

Available Diagnostic Approaches for NTS

For culture-based and molecular diagnostics, similar challenges present with typhoidal Salmonella—low bacterial burden (1CFU/ml) in blood—may confound sensitive diagnosis of NTS [152]. PCR assays have been clinically validated for diagnosis of enterocolitis or for use on cultured isolates, but not for direct diagnosis of invasive infections [153,154].

In contrast with typhoidal Salmonella, there is more limited experience with serologic assays for NTS. Despite similarity between the O antigens of *S. typhi* and *S. enteritidis,* the Widal agglutination test performs poorly [155]. Kuhn et al. recently reviewed ELISAs for *non-typhoidal Salmonella* infections, which were based on *S. enteritidis* and *S. typhimurium* LPS, and found most sensitivities in the 70–95% range, with specificities >90%, though most studies were small and many did not report specificity [156].

Conclusion

Typhoid fever is having a past of several sporadic outbreaks and is found to be endemic in several nations. Due to better sanitation facilities, hygienic life style, clean food and water resources and mass vaccination for *Salmonella typhi*, the typhoid has been almost eradicated from developed countries. But in developing countries like India, these facilities cannot be availed by a major part of population so enteric fever has been a constant problem.

None of the currently available typhoid vaccines are ideal for enteric fever. The first line of parenteral whole cell vaccines has been associated with fever and systemic reactions. Although licensed, it is considered unsuitable for mass immunization and no longer in use [157]. Consequently two vaccines have

been developed. The first is a live oral vaccine based on Ty21a (an attenuated strain of S. typhi) that is well-tolerated and the other is Vi-based parenteral subunit vaccine (based on the purified capsular polysaccharide *S. typhi* Vi antigen). Both these vaccines are well tolerated but are only moderately protective [158]. Controlled, double blind, randomized field trials of the Vi typhoid polysaccharides had demonstrated the protection against the typhoid fever in areas with higher endemicity. The Vi vaccine has been targeted for accelerated introduction into public health programs, due to several advantages it has over Ty21a, including consistent efficacy results (64-77%) even in areas of high typhoid incidence; a single-dose regimen; the lack of patent protection; and less strict cold chain requirements [101]. The typhoid Vi-conjugate vaccine can be used for the immunization of the children, adult and travellers from non endemic areas and for people living in endemic countries.

Laboratory diagnosis of enteric fever in developing countries is primarily achieved either by blood culture or bone marrow aspirate culture which are gold standard for diagnosis. The diagnosis of typhoid fever based on clinical signs and symptoms is often ambiguous, while phenotypic detection of *S. typhi* bacteria based on biochemical and serotyping methods is laborious and time consuming [17]. Due to invasiveness and technical difficulty of the procedure, one has to rely on serological diagnosis. Unfortunately, neither the Widal test, nor any of the serodiagnostic tests that have since been developed have proven sufficiently sensitive, specific and practical to be of value in areas where this disease is endemic [4]. For the detection of convalescent and chronic fecal carriage of typhoidal Salmonella, and to estimate the burden of disease for public health assessment. Different tests and biological samples may be required for each situation. It may be important to be able to detect both *Salmonella serovar typhi* and *Salmonella serovar Paratyphi A* infections, as they cannot be distinguished from each other clinically. Microbial culture is the mainstay of diagnosis [86]. The challenge in any diagnostic studies is to reach to a correct final diagnosis in all participants. Usually a single error-free reference test, known as a gold standard, is used to determine the final diagnosis and estimate the accuracy of the test under evaluation. In disease where there is no failsafe method for diagnosis, a Composite Reference Standard (CRS) is used to make a final diagnosis based on the results of two or more diagnostic tests known as component tests. Patients are subjected to same component tests which are combined and interpreted in a fixed way for all patients which reflects the presence or absence of the target disease.

Antigen based rapid diagnostic test kits in particular could potentially save lives, time and money. Rapid detection methods, including multiplex PCR and stool dipstick tests as well as a fast blood culture PCR method, are being developed to aid early diagnosis. Newer assays are rarely available in developing countries [159].

Although Tubex and Typhidot M tests are being available and widely used in the clinical laboratories, there is a need for a rapid and in expensive laboratory test for early and accurate diagnosis of patients with typhoid fever has prompted the exploration of a variety of serologic and antigen detection methods. The assays like counter immuno-electrophoresis, Enzyme-Linked Immunosorbent assay (ELISA), dot immunoassay, hemagglutination and coagglutination are not very easy to perform, not rapid, require special equipment or skills, or depend on electricity and on refrigeration for storage of components. None of these assays has yet reached widespread use.

Because of the lack of the availability of good diagnostic tools, particularly that are field based, correct diagnosis of typhoid fevers remains a grey area. So the researchers in the developing countries should emphasis the research on the development of newer and easy diagnostic assays using various antigens of *Salmonella typhi.*

References

1. Nataro Jp, Cheryl A. Bopp, Patricia I. Fields, James B. Kaper, Nancy A. Strockbine. Escherichia; Shigella; Salmonella. Manual of Clinical Microbiology. 2011;10:603-626. doi: 10.1128/9781555816728
2. Wain J, House D, Parkhill J, Parry C, Dougan G. Unlocking the genome of human typhoid bacillus. Lancet Infect Dis. 2002;2(3):163-170
3. Haque A. Utility of PCR in diagnosis of problematic cases of typhoid. Jpn. J. Infect. Dis. 2001;54:237-239
4. Maheshwari V, Navinchandra Motiram Kaore, Vijay Kumar Ramnani, Sheil Sarda. A Comparative Evaluation of Different Diagnostic Modalities in the Diagnosis of Typhoid Fever using a composite reference standard: A Tertiary Hospital based study in central India. J Clin Diagn Res. 2016;10(10):DC01-DC04. doi: 10.7860/JCDR/2016/20426.8684
5. Hessel L, Debois H, Fletcher M, Dumas R. Experience with *Salmonella typhi* Vi capsular polysaccharide vaccine. Eur J Clin Microbiol Infect Dis. 1999;189(9):609-620
6. Haque A, Ahmed J, Qureshi JA. Early detection of typhoid by polymerase chain reaction. Ann Saudi Med. 1999;19(4):337-340
7. Zaki SA, Karande S. Multidrug-resistant typhoid fever: a review. J Infect Dev Ctries. 2011;5(5): 324-337
8. Ugochukwu I, Amu OC, Nzegwu MA. Ileal perforation due to typhoid fever—review of operative management and outcome in an urban centre in Nigeria. Int J Surg. 2013;11(3):218-222
9. Sandro Contini. Typhoid intestinal perforation in developing countries: Still unavoidable deaths?. World J Gastroenterol. 2017;23(11):1925-1931. doi: 10.3748/wjg.v23.i11.1925
10. Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. Lancet. 2005;336(9487):749-762. doi: 10.1016/S0140-6736(05)67181-4
11. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ. 2006;333(7558):78–82
12. Kundu R, Ganguly N, Ghosh TK, Yewale VN, Shah RC, Shah NK, et al. IAP Task Force Report: diagnosis of enteric fever in children. Indian Pediatr. 2006;43(10):875-883
13. Wain J, Hosoglu S. The laboratory diagnosis of enteric fever. J Infect Dev Ctries. 2008;2(6):421-425
14. Parry CM, Wijedoru L, Arjyal A, Baker S. The utility of diagnostic tests for enteric fever in endemic locations. Expert Rev Anti Infect Ther. 2011;9(6):711-725. doi: 10.1586/eri.11.47
15. Ananthanarayan, Panikar. Textbook of Microbiology. Chapter 32,

Enterobacteriacea III-Salmonella; 2005;288-289

16. Brenner DJ, Kreig NR, Stanley JT. Genus. Salmonella. In: Bergeys Manual of systematic bacteriology. 2005;2(2):764-799. New York.

17. Yuan Xin Goay, Kai Ling Chin, Clarissa Ling Ling Tan, Chiann YingYeoh, Ja'afar Nuhu Ja'afar, Abdul Rahman Zaidah, et al. Identification of Five Novel *Salmonella typhi*-Specific Genes as Markers for Diagnosis of Typhoid Fever Using Single-Gene Target PCR Assays. BioMed Research International. 2016;1-9. doi: 10.1155/2016/8905675

18. Grimont PAD, Weill FX. Antigenic formulae of the Salmonella serovars-2007. WHO collaborating Centre for Reference and Research on Salmonella. 9th EDN. Institute Pastuer; Paris: France: 2007.

19. Gabrielle MH, Curtis N. *Non-typhoidal Salmonella* in Children: Microbiology, Epidemiology and Treatment Hot Topics in Infection and Immunity in Children, Advances in Experimental Medicine and Biology. 2013;764. doi 10.1007/978-1-4614-4726-9_2

20. Hiriart, Maria Serradell, Araci Martínez, Sofia Sampaolesi, Dolores Gonzalez Maciel, Jose Alejandro, et al. Generation and selection of anti-flagellin monoclonal antibodies useful for serotyping Salmonella enteric. Springer Plus. 2013;2(640):1-9. doi: 10.1186/2193-1801-2-640

21. Ramos HC, Rumbo M, Sirard JC. Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. Trends Microbiol. 2004;12(11):509–517

22. Threlfall EJ. Topley and Wilson Microbiology and microbial infection bacteriology. 10th edition; 2007;541:398-1427

23. Brenner FW, R. G. Villar, F. J. Angulo, R. Tauxe, B. Swaminathan. Salmonella nomenclature. J Clin Microbiol. 2000;38(7):2465–2467

24. Gray SB, J H Green , W K Harrell , L Britt , R C Bolin. Reuse of Salmonella and Shigella absorbing cells for preparing monospecific Salmonella O and Shigella antisera. Appl Microbiol. 1974;28(2):320–322

25. Ibrahim GF, G H Fleet , M J Lyons , R A Walker. Immunological relationships between Salmonella flagella and their potential application for salmonellae detection by immunoassay. Med Microbiol Immunol. 1985;174(2):87–99

26. Ibrahim GF, Fleet GH, Lyons MJ, Walker RA. Production of potent Salmonella H antisera by immunization with polymeric flagellins. J Clin Microbiol. 1985;22(3):347-351

27. Rementeria A, Vivanco AB, Ramirez A, Hernando FL, Bikandi J, Herrera-León S, et al. Characterization of a monoclonal antibody directed against *Salmonella enterica serovar typhimurium* and serovar [4,5,12:i:-]. Appl Environ Microbiol. 2009;75(5):1345-1354. doi: 10.1128/AEM.01597-08

28. Iankov ID, Petrov DP, Mladenov IV, Haralambieva IH, Ivanova R, R Velev V, et al. Production and characterization of monoclonal immunoglobulin A antibodies directed against Salmonella H:g,m flagellar antigen. FEMS Immunol Med Microbiol. 2002;33(2):107–113

29. Nelson PN, Reynolds GM, Waldron EE, Ward E, Giannopoulos K, Murray PG. Monoclonal antibodies. Mol Pathol. 2000;53(3):111–117

30. Kintz, Christian Heissb, Ian Blackb, Nicholas Donohuea, Naj Browna, Mark R. Daviesa, et al. Salmonella enterica Serovar typhi Lipopolysaccharide O-Antigen Modification Impact on Serum Resistance and Antibody Recognition. Amrican society for Microbiology: Infection and Immunity. 2017;85(4):1-10. doi: 10.1128/IAI.01021-16

31. Silliker JH, Gabis DA. Laboratory diagnosis of infectious disease principle and practice. I Bacterial mycotic and Parasitic disease 1988;47:448-464

32. Murray GL, Stephen R. Attridg, Renato Morona. Altering the length of the lipopolysaccharide O antigen has an impact on the interaction of *Salmonella enterica serovar Typhimurium* with macrophages and complement. J Bacteriol. 2006;188(7):2735–2739. doi: 10.1128/JB.188.7.2735–2739.2006

33. MacLennan CA. Antibodies and protection against invasive salmonella disease. Front Immunol. 2014;5:635. Doi: 10.3389/fimmu.2014.00635

34. Nagy G, Pal T. Lipopolysaccharide: a tool and target in enterobacterial vaccine development. Biol Chem. 2008;389(5):513–520

35. Zhu Q, Lim CK, Chan YN. Detection of *Salmonella typhi* by polymerase chain reaction. J Appl Bacteriol. 1996;80(3):244–251

36. Hone DM, Harris AM, Chatfield S, Dougan G, Levine MM. Construction of genetically defined double aro mutants of *Salmonella typhi*. Vaccine. 1991;9(11):810–816

37. House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay PV, et al. Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. J Clin Microbiol. 2001;39(3):1002–1007. doi: 10.1128/JCM.39.3.1002-1007.2001

38. Kantele A, Pakkanen SH, Siitonen A, Karttunen R, Kantele JM. Live oral typhoid vaccine *Salmonella typhi* Ty21a—a surrogate vaccine against Non-typhoid salmonella?. Vaccine. 2012;30(50):7238 –7245. doi: 10.1016/j.vaccine.2012.10.002

39. Wain J, Deborah House, Afia Zafar, Stephen Baker, Satheesh Nair, Claire Kidgell, et al. Vi antigen expression in Salmonella enterica serovar typhi clinical isolates from Pakistan. J Clin Microbiol. 2005;43(3):1158–1165

40. Hohmann EL. David Acheson. Non-typhoidal Salmonellas is. Clin Infect Dis.2001;32(2):263–269. doi: 10.1086/318457

41. Marshall JM, Gunn JS. The O-Antigen Capsule of *Salmonella enterica Serovar Typhimurium* Facilitates Serum Resistance and Surface Expression of FliC. Infect Immun. 2015;83(10):3946-3959. doi: 10.1128/IAI.00634-15

42. Giannella, Ralph A. Medical Microbiology. 4th edition, Edited by s Baron. The University of Texas Medical Branch at Galveston. 1966.

43. Kothari A, Pruthi A, Chugh TD. The burden of enteric fever. J Inft Dev Ctries. 2008;2(4):253-259

44. Crump JA, Heyderman RS. A Perspective on Invasive Salmonella Disease in Africa. Clin Infect Dis. 2015;61 Suppl 4:S235-S240. doi: 10.1093/cid/civ709

45. Kumar, N. Gupta, V.B. Vaish, S. Gupta. Distribution trends & antibiogram pattern of Salmonella enteric serovar Newport in India. Indian J Med Res. 2016;144(1):82-86. doi: 10.4103/0971-5916.193293

46. Osler W. The principles and practice of medicine: designed for the use of practitioners and students of medicine. 8th ed. D. Appleton and Company, New York: NY; 1901.

47. Stuart BM, Pullen RL. Typhoid: clinical analysis of 360 cases. Arch Intern Med.1946;78(6):629–661

48. Huckstep RL. Typhoid fever and other Salmonella infections. 1962;77(5):311-316. Livingstone, Edinburgh; Scotland.

49. Lin FYC, Vo AH, Phan VB, Nguyen TT, Bryla D, Tran CT, et al. The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. Am J Trop Med Hyg. 2000;62(5):644–648

50. 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(9180):734–737

51. Kasper MR, Buth Sokhal, Patrick Blair, Shan Putnam. Emergence of multidrug-resistant Salmonella enterica serovar typhi with reduced susceptibility to fluoroquinolones in Cambodia. Diagn Microbiol Infect Dis. 2009;66(2):207–209

52. Ferreccio C, Levine MM, Manterola A, Rodriguez G, Rivara I, Prenzel I, et al. Benign bacteremia caused by *Salmonella typhi* and Paratyphi in children aged younger than 2 years. J Pediatr. 1984;104(6):899–901

53. Topley JM. Mild typhoid fever. Arch Dis Child. 1986;61(2):164–167

54. Mahle WT, Levine MM. *Salmonella typhi* infection in children younger than five years of age. Pediatr Infect Dis J. 1993;12(8):627–631

55. Waddington CS, Darton TC, Pollard AJ. The challenge of enteric fever. J Infect. 2014;68 Suppl 1:S38 –S50. doi: 10.1016/j.jinf.2013.09.013

56. Waddington CS, Darton TC, Woodward WE, Angus B, Levine MM, Pollard AJ. Advancing the management and control of typhoid fever: a review of the historical role of human challenge studies. J Infect. 2014;68(5):405–418. doi: 10.1016/j.jinf.2014.01.006

57. Waddington CS, Darton TC, Jones C, Haworth K, Peters A, John T, et al. An outpatient, ambulant-design, controlled human infection model using escalating doses of *Salmonella typhi* challenge delivered in sodium bicarbonate solution. Clin Infect Dis. 2014;58(9):1230–1240. doi: 10.1093/cid/ciu078

58. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347(22):1770–1782. doi: 10.1056/NEJMra020201

59. Cunha BA, Sigridh Munoz-Gomez, Arthur Gran. Typhoid fever vs. malaria in a febrile returning traveler: typho malaria revisited: an Oslerian perspective. Travel Med Infect Dis. 2013;11(4):66–69. doi: 10.1016/j.tmaid.2012.09.003

60. Crump, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev. 2015;28(4):901-937. doi: 10.1128/CMR.00002-15

61. P.K. Pandove, Ashish Moudgil, Megha Pandove, Kamna Aggarwal, Divya Sharda, Vijay K. Sharda. Multiple ileal perforations and concomitant cholecystitis with gall bladder gangrene as complication of typhoid fever. J Surg Case Rep. 2014(7):rju070 doi: 10.1093/jscr/rju070

62. Ameh EA. Typhoid ileal perforation in children: a scourge in developing countries. Ann Trop Paediatr. 1999;19(3): 267–272

63. Song JH , Cho H, Park MY, Na DS, Moon HB, Pai CH. Detection of *Salmonella typhi* in the blood of patients with typhoid fever by polymerase chain reaction. J Clin Microbiol. 1993;31(6):1439–1443

64. Mogasale, Desai SN, Mogasale VV, Park JK, Ochiai RL, Wierzba TF. Case Fatality Rate and Length of Hospital Stay among patients with Typhoid Intestinal Perforation in Developing Countries: A Systematic Literature Review . Plos one. 2014;9(4): e93784. doi: 10.1371/journal.pone.0093784

65. WHO Background document: The diagnosis, treatment and prevention of typhoid fever. World Health Organization. 2003:Geneva, Switzerland

66. Saxena V, Basu S, Sharma CL. Perforation of the gall bladder following typhoid fever induced ileal perforation. Hong kong Med J. 2007;13(6):475–477

67. Uba AF, Chirdan LB, Ituen AM, Mohammed AM. Typhoid intestinal perforation in children: a continuing scourge in a developing country. Pediatr Surg Int. 2007;23(1):33–39. doi: 10.1007/s00383-006-1796-3

68. Rahman GA, Abubakar AM, Johnson AW, Adeniran JO. Typhoid ileal perforation in Nigerian children: an analysis of 106 operative cases. Pediatr Surg Int . 2001;17(8):628–630. doi: 10.1007/s003830100008

69. Schulte M, Hensel M. Models of intestinal infection by Salmonella enterica: introduction of a new neonate mouse model version. F1000 Research. 2016;5(1498):1-8. doi:10.12688/f1000research.8468.1

70. NizamiSQ, Bhutta ZA, Siddiqui AA, Lubbad L. Enhanced detection rate of typhoid fever in children in a peri urban slum in Karachi, Pakistan using polymerase chain reaction technology. Scand J Clin Lab Invest. 2006;66(5):429–436

71. Rosenberger CM, Scott MG, Gold MR, Hancock RE, Finlay BB. *Salmonella typhi*murium infections and lipopolysaccharides stimulation induce similar changes in macrophage gene expression. J. immunol. 2000;164(11):5894-5904

72. Oanh HP, Stephen JM: Protective host immune responses to Salmonella infection. Future Microbiol. 2015;10:101–110. doi: 10.2217/fmb.14.98

73. Everest P, Wain J, Roberts M, Rook G, Dougan G. The molecular mechanism of severe typhoid fever. Trends Microbiol. 2001;9(7):316-320

74. Bryan C, Guntram AG, Finlay BB. Salmonella, the host and disease: a brief review. Immunol Cell Biol. 2007;85(2):112–118.

75. Bhattacharya SS, Das U, Chaudhury BK. Occurrence and antibiogram of *Salmonella typhi* and S. Paratyphi A isolated from Rourkela, Orissa. Indian J Med Res. 2011;133(4):431-433

76. Crump, Mintz ED. Global trends in typhoid and paratyphoid fever. Clin Infect Dis. 2010;50(2):241-246. doi: 10.1086/649541

77. Imran ZP, Anita Zaidi, Anwer Ali Siddiqui, Satheesh Nair, Aqeel Ahmadet. Molecular and epidemiological analyses of multidrug-resistant Salmonella para typhi A obtained from different regions of Pakistan. Pak. J. Bot. 2010;42(4):2941-2947

78. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ. 2006;333(7558):78-82

79. Kariuki S, C. Gilks, G. Revathi, C. A. Hart. Genotypic analysis of multidrug-resistant Salmonella enterica serovar typhi, Kenya. Emerg Infect Dis. 2000;6(6):649–651. doi: 10.3201/eid0606.000616

80. Lewis MD, Serichantalergs O, Pitarangsi C, Chuanak N, Mason CJ, Regmi LR, et al. Typhoid Fever: A Massive, Single-Point Source, Multidrug-Resistant Outbreak in Nepal. Clin Infect Dis. 2005;40(4):554–561

81. M Yan, Xinlan Li, Qiaohong Liao, Fang Li, Jing Zhang, Biao Kan. The emergence and outbreak of multidrug-resistant typhoid fever in China. Emerging Microbes and Infections. 2016;5(e62):1-6. doi:10.1038/emi.2016.62

82. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and Non-typhoidal Salmonellae. Curr Opin Infect Dis. 2008;21(5):531–38. doi: 10.1097/QCO.0b013e32830f453a

83. Chen HM, et al. Non-typhoid Salmonella infection: microbiology, clinical features, and antimicrobial therapy. Pediatr Neonatol. 2013;54(3):147–52. doi: 10.1016/j.pedneo.2013.01.010

84. Antunes, Mourão J, Campos J, Peixe L. Salmonellosis: the role of poultry meat. Clin Microbiol Infect. 2016;22(2):110-121. doi: 10.1016/j.cmi.2015.12.004

85. Jneid, Karine Moreau, Marc Plaisance, Audrey Rouaix, Julie Dano, Stéphanie Simon. Role of T3SS-1 SipD Protein in Protecting Mice

against *Non-typhoidal Salmonella Typhi*murium. PLoS Negl Trop Dis. 2016;10(12): e0005207. doi: 10.1371/journal.pntd.0005207

86. Crump JA, Maria Sjölund-Karlsson, Melita A. Gordon, Christopher M. Parry. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive Salmonella infections. Clin Microbiol Rev. 2015;28(4)901-937. doi:10.1128/CMR.00002-15

87. Cianflone NF. Salmonellosis and the gastrointestinal tract: more than just peanut butter. Curr Gastroenterol Rep. 2008;10(4):424-431

88. Galofré J, Moreno A, Mensa J, Miró JM, Gatell JM, Almela M, et al. Analysis of factors influencing the outcome and development of septic metastasis or relapse in Salmonella bacteremia. Clin Infect Dis.1994;18(6):873-878

89. Kucuk O. Report of data on children with non-typhi Salmonella gastroenteritis in a three-year period. BioPortfolio. 2016;194-200.

90. Yurdakök K, Asaker EA, Berkman E. Salmonella gastroenteritis in children. Turk J Pediatr. 1998;40(1):69-78

91. Kizirgil A, Karakoç S. Detection of the ethiologic agents of the childhood acute gastroenteritis. Nobel Med. 2012;8(3):60-65

92. Mogasale V, Maskery B, Ochiai RL, Lee JS, Mogasale VV, Ramani E, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health. 2014;2(10):e570–e580. doi: 10.1016/S2214-109X(14)70301-8

93. Arndt MB, Mosites EM, Tian M, Forouzanfar MH, Mokhdad AH, Meller M, et al. Estimating the burden of paratyphoid a in Asia and Africa. PLoS Negl Trop Dis. 2014;8(5):e2925. doi: 10.1371/journal.pntd.0002925

94. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10(6):417–432. doi: 10.1016/S1473-3099(10)70072-4

95. Andrews JR, Ryan ET. Diagnostics for invasive Salmonella infections: current challenges and future directions. Vaccine. 2015;33(0 3):C8-C151-18. doi:10.1016/j.vaccine.2015.02.030

96. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2197–2223. doi: 10.1016/S0140-6736(12)61689-4

97. Dewan, Corner R, Hashizume M, Ongee E. Typhoid Fever and Its Association with Environmental Factors in the Dhaka Metropolitan Area of Bangladesh: A Spatial and Time-Series Approach. PLoS Negl Trop Dis. 2013;7(1):e1998. doi: 10.1371/journal.pntd.0001998

98. Watson CH, Edmunds WJ. A review of typhoid fever transmission dynamic models and economic evaluations of vaccination. Vaccine. 2015; 33 Suppl 3:C42-54. doi: 10.1016/j.vaccine.2015.04.013

99. Kanj SS, Kanafani ZA, Shehab M, Sidani N, Baban T, Baltajian K, et al. Epidemiology, clinical manifestations, and molecular typing of *Salmonella typhi* isolated from patients with typhoid fever in Lebanon . J Epidemiol Glob Health. 2015;5(2):159– 165. doi: 10.1016/j.jegh.2014.07.003

100. Pham OH, Mc Sorley SJ. Protective host immune responses to Salmonella infection. Future Microbiol. 2015;10:101–110. doi: 10.2217/fmb.14.98

101. Kanungo, Dutta S, Sur D. Epidemiology of typhoid and paratyphoid fever in India. J Infect Dev Ctries. 2008;2(6):454-460

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

103. Grabenstein JD, Pittman PR, Greenwood JT, Engler RJ. Immunization to protect the US Armed Forces: heritage, current practice, and prospects. Epidemiol Rev. 2006;28:03-26

104. Galan JE. Typhoid toxin provides a window into typhoid fever and the biology of *Salmonella Typhi*. Proc Natl Acad Sci U S A. 2016;113(23):6338-6344. doi: 10.1073/pnas.1606335113

105. Galen JE, Curtiss R. The delicate balance in genetically engineering live vaccines. Vaccine. 2014;32(35):4376–4385. doi: 10.1016/j.vaccine.2013.12.026

106. Levine MM, Ferreccio C, Cryz S, Ortiz E. Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in randomised controlled field trial. Lancet. 1990;336(8720):891–894

107. World Health Organization. Typhoid vaccines: WHO position paper .Wkly Epidemiol Rec. 2008;83(6):49–59

108. Cumming O, Elliott M, Overbo A, Bartram J. Does global progress on sanitation really lag behind water? An analysis of global progress on community and household level access to safe water and sanitation. PLoS One. 2014;9(12):e114699. doi: 10.1371/journal.pone.0114699

109. Pasetti MF, Simon JK, Sztein MB, Levine MM. Immunology of gut mucosal vaccines. Immunol Rev. 2011;239(1):125–148. doi: 10.1111/j.1600-065X.2010.00970.x

110. Mestecky J, Nguyen H, Czerkinsky C, Kiyono H. Oral immunization: an update. Curr Opin Gastroenterol. 2008;24(6):713–719. doi: 10.1097/MOG.0b013e32830d58be

111. Olanratmanee T, Levine M, Losonsky G, Thisyakorn V, Cryz SJ Jr, et al. Safety and immunogenicity of *Salmonella typhi* Ty21a liquid formulation vaccine in 4- to 6-year-old Thai children. J Infect Dis. 1992;166(2):451–452

112. Sur D, Ochiai RL, Bhattacharya SK, Ganguly NK, Ali M, Manna B, et al. A cluster-randomized effectiveness trial of Vi typhoid vaccine in India. N Engl J Med.2009;361(4):335–344. doi: 10.1056/NEJMoa0807521

113. Ali A, Haque A, Haque A, Sarwar Y, Mohsin M, Bashir S, et al. Multiplex PCR for differential diagnosis of emerging Typhoidal pathogens directly from blood samples. Epidemiol Infect. 2009;137(1):102–107. doi: 10.1017/S0950268808000654

114. World Health Organization, Technical Report Series, No. 840, (1994) :16.

115. Ruan R, Xia XP, Sun D, Ojcius DM, Mao YF, Yue WY, et al. Recombinant Spao and H1a as immunogens for protection of mice from lethal infection with Salmonella paratyphi A: implications for rational design of typhoid fever vaccines. Vaccine. 2008;26(51): 6639-6644. doi: 10.1016/j.vaccine.2008.09.030

116. Roland KL et.al. Reactogenicity and immunogenicity of live attenuated Salmonella enterica Paratyphi A enteric fever vaccine candidates. Vaccine. 2010;28(21):3674-3687. doi: 10.1016/j.vaccine.2010.03.019

117. 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(1):78-83. doi: 10.3892/mmr.2011.587

118. Gat O, Galen JE, Tennant S, Simon R, Blackwelder WC, Silverman DJ, et. al. Cell- associated flagella enhance the protection conferred by mucosally- administered, attenuated Salmonella paratyphi A vaccines. PLoS Negl Trop Dis. 2011;5(11): :e1373. doi: 10.1371/journal.pntd.0001373

119.Konadu E, Robbins JB, Shiloach J, Bryla DA, Szu SC. Preparation, Characterization and Immunological properties in mice of Esherichia coli O157 O-specific polysaccharide protein conjugate vaccines. Infect Immun. 1994;62(11):5048-5054

120.Konadu E, J Shiloach, D A Bryla, J B Robbins, S C Szu, et al. Synthesis, Characterization and Immunological properties in mice of conjugates composed of detoxified lipopolysacharide of Salmonella paratyphi A bound to tetanus toxoid with emphasis on the role of O-acetyls. Infect Immun. 1996;64(7):2709-2715

121.Widal GG, Scicard A: Bull. Mem. Soc. Med. Hop. Paris 3 ser, vol. 13, pp-681. Cited : Paterson JCS (1949) Typhoid and paratyphoid: A review. Post grad. Med.J.,25, 413-420

122.Chart H, Cheasty T, de Pinna E, Siorvanes L, Wain J, Alam D, et al. Serodiagnosis of Salmonella enterica serovar typhi and S. enterica serovars Paratyphi A, B and C human infections. Journal of Medical Microbiology. 2007;56(pt 9):1161–1166

123.Mogasale V, Enusa Ramani, Vijayalaxmi V, Mogasale, JuYeon Park, et al. What proportion of *Salmonella Typhi* cases are detected by blood culture? A systematic literature review. Ann Clin Microbiol Antimicrob. 2016;15:32. doi: 10.1186/s12941-016-0147-z

124.Biswas R et al. An unusual paratyphoid fever. J Assoc Physicians India. 2001;49:477-478

125.Rubin FA, Mc Whirter PD, Punjabi NH, Lane E, Sudarmono P, Pulungsih SP, et al. Use of a DNA probe to detect *Salmonella typhi* in the blood of patients with typhoid fever. J Clin Microbiol. 1989;27(5):1112–1114

126.Kamat SA, Herzog C. Typhoid: clinical picture and response to chloramphenicol. Prospective study in Bombay (1972). Infection. 1977;5(2):85–91

127.Stuart BM, Pullen RL. Typhoid; Clinical analysis of three hundred and sixty cases. Arch Intern Med. 1946;78(6):629–661

128.Hatta M, Goris MG, Heerkens E, Gooskens J, Smits HL. Simple Dipstick Assay For The Detection Of *Salmonella Typhi*-Specific Igm Antibodies And The Evolution Of The Immune Response In Patients With Typhoid Fever. Am J Trop Med Hyg. 2002;66(4):416–421

129.Gasem MH, Dolmans WM, Isbandrio BB, Wahyono H, Keuter M, Djokomoeljanto R. Culture of *Salmonella typhi* and Salmonella Paratyphi from blood and bone marrow in suspected typhoid fever. Trop Geogr Med. 1995;47(4):164–167

130.Andrews JR, Ryan ET. Diagnostics for invasive Salmonella infections: current challenges and future directions. Vaccine. 2015;33 Suppl 3:C8-15. doi: 10.1016/j.vaccine.2015.02.030

131.Wain J, Diep TS, Ho VA, Walsh AM, Nguyen TT, Parry CM, et al. Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. J. Clin. Microbiol. 1998;36(6):1683–1687.

132.Watson KC, Laurie W. The laboratory diagnosis of typhoid fever in areas of endemicity. Am J Trop Med Hyg. 1956;5(6):1051–1057. doi: 10.4269/ajtmh.1956.5.1051

133.Watson K.C. Isolation of *Salmonella Typhi* from the bloodstream. J Lab Clin Med. 1955;46(1):128–134

134.Sénchez-Jiménez MM, Cardona-Castro N. Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the hilA gene in clinical samples from Colombian patients. J Med Microbiol. 2004;53(pt 9):875–878. doi: 10.1099/jmm.0.45630-0

135.Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. Lancet. 1975;1(7918):1211–1213

136.Wain J, Pham VB, Ha V, Nguyen NM, To SD, Walsh AL, et al. Quantitation of Bacteria in Bone Marrow from Patients with Typhoid Fever: Relationship between Counts and Clinical Features. J Clin Microbiol. 2001;39(4):1571–1576. doi: 10.1128/JCM.39.4.1571-1576.2001

137.Aziah I, Ravichandran M, Ismail A. Amplification of ST50 gene using dry-reagent-based polymerase chain reaction for the detection of *Salmonella Typhi*. Diagn Microbiol Infect Dis. 2007;59(4):373–377. doi: 10.1016/j.diagmicrobio.2007.05.014

138.Kumar A, Balachandran Y, Gupta S, Khare S, Suman. Quick PCR based diagnosis of typhoid using specific genetic markers. Biotechnol Lett. 2010;32(5):707–712. doi: 10.1007/s10529-010-0211-2

139.G. Frankel, Newton SM, Schoolnik GK, Stocker BA. Intragenic recombination in a flagellin gene: characterization of the H1-j gene of *Salmonella typhi*. EMBO J. 1989;8(10): 3149–3152

140.Gasem MH, Smits HL, Goris MG, Dolmans WM. Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia. J Med Microbiol. 2002;51(2):173-177

141.Baker S, Abhilasha Karkey, Christopher Parry. Are we adequately prepared for the emergence of Salmonella enteric serovar Paratyphi A?. The Lancet. 2014;2(4):e195-e196. doi: 10.1016/S2214-109X(14)70009-9

142.Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant *Salmonella typhi*: a worldwide epidemic. Clin Infect Dis. Suppl 1:S106-9

143.Renuka K, Sood S, Das BK, Kapil A. High-level ciprofloxacin resistance in Salmonella enterica serotype Typhi in India. J Med Microbiology. 2005;54(pt 10):999-1000. doi: 10.1099/jmm.0.45966-0

144.K.Hirose, Ken-Ichiro Itoh, Hiroshi Nakajima, Takayuki Kurazono, Masanori Yamaguchi, Kazuo Moriya, et al. Selective amplification of tyv (rfbE), prt (rfbS), viaB, and fliC genes by multiplex PCR for identification of Salmonella enteric serovars Typhi and Paratyphi A. J Clin Microbiol. 2002;40(2):633–636. doi: 10.1128/JCM.40.2.633-636.2002

145.Castro NC, Fmrex PA. Immuno-enzymatic dot-blot test for the diagnosis of enteric fever caused by *Salmonella typhi* in an endemic area. Clinical Microbiology &Infec. 1998;4(2):64-69. doi: 10.1111/j.1469-0691.1998.tb00357.x.

146.Choo KE, Oppenheimer SJ, Ismail AB, Ong KH. Rapid serodiagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. Clin Infect Dis. 1994;19(1):172-176

147.Chaicumpa W, Ngren-ngarmlert W, Kalambaheti T, Ruangkunaporn Y, Chongsa-nguan M, Tapchaisri P, et al. Monoclonal Antibody-Based Dot-Blot ELISA for the Detection of Salmonella in Foods. Asian Pac J Allergy Immunol. 1995;13(2):159-166

148.H. M. Seth-Smith, SPI-7: Salmonella's Vi-encoding Pathogenicity Island. Journal of Infection in Developing Countries. 2008;2(4): 267–271

149.Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, et al. Identification by PCR of *Non-typhoidal Salmonella* enteric Serovars Associated with Invasive Infections among Febrile Patients in Mali. PLoS Negl Trop Dis. 2010; 9;4(3):e621. doi: 10.1371/journal.pntd.0000621.

150.Ley B, Mtove G, Thriemer K, Amos B, von Seidlein L, Hendriksen I, et al. Evaluation of the Widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hospital in Tanzania

and a comparison with previous studies. BMC Infect Dis. 2010;10:180. doi: 10.1186/1471-2334-10-180.

151.Lunguya O, Phoba MF, Mundeke SA, Bonebe E, Mukadi P, Muyembe JJ, et al. The diagnosis of typhoid fever in the Democratic Republic of the Congo. Trans R Soc Trop Med Hyg. 2012;106(6):348–355. doi: 10.1016/j.trstmh.2012.03.006.

152.Gordon MA, Kankwatira AM, Mwafulirwa G, Walsh AL, Hopkins MJ, Parry CM, et al. Invasive non-typhoid salmonellae establish systemic intracellular infection in HIV-infected adults: an emerging disease pathogenesis. Clin Infect Dis. 2010;50(7):953–962. doi: 10.1086/651080.

153.Lin L-H, Tsai CY, Hung MH, Fang YT, Ling QD. Rectal swab sampling followed by an enrichment culture-based real-time PCR assay to detect Salmonella enterocolitis in children. Clin Microbiol Infect. 2011;17(9):1421–1425. doi: 10.1111/j.1469-0691.2010.03450.x.

154.Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, et al. Identification by PCR of *Non-typhoidal Salmonella* enteric Serovars Associated with Invasive Infections among Febrile Patients in Mali. PLoS Negl Trop Dis. 2010;4(3):e621. doi: 10.1371/journal.pntd.0000621.

155.Strid MA, Tine Dalby, Kåre Mølbak, Karen A. Krogfelt. Kinetics of the human antibody response against Salmonella entericaSerovarsEnteritidis and Typhimurium determined by lipopolysaccharide enzyme-linked immunosorbent assay. Clin Vaccine Immunol. 2007;14(6):741–747. doi: 10.1128/CVI.00192-06.

156.Kuhn KG, Falkenhorst G, Ceper TH, Dalby T, Ethelberg S, Mølbak K, et al. Detecting non-typhoid Salmonella in humans by ELISAs: a literature review. J Med Microbiol. 2012;61(pt 1):1–7. doi: 10.1099/jmm.0.034447-0.

157.Garmony HS, Brown KA, Titball RW. Salmonella vaccines for use in humans: present and future perspectives. FEMS Microbiol Rev. 2002;26(4):339–353.

158.Kaur J, Jain SK. Role of antigens and virulence factors of Salmonella enteric serovar Typhi in its pathogenesis. Microbiol Res. 2012;167(4):199– 210. doi: 10.1016/j.micres.2011.08.001.

159.Meiying Y, Bo Yang, Zhigang Wang, Shukun Wang, Xiaohe Zhang, Yanhua Zhou, et al. A Large-Scale Community-Based Outbreak of Paratyphoid Fever Caused by Hospital- Derived Transmission in Southern China. PLoS Negl Trop Dis. 2015;1-14. doi: 10.1371/journal.pntd.0003859.

160.Pratap CB, Kumar G, Patel SK, Verma AK, Shukla VK, Kumar K, et al. Targeting of putative fimbrial gene for detection of S. typhi in typhoid fever and chronic typhoid carriers by nested PCR. J Infect Dev Ctries. 2013;7(7):520–527. doi: 10.3855/jidc.2561

161.Yin Ngan GJ, . Development of a novel multiplex PCR for the detection and differentiation of Salmonella enterica serovars typhi and Paratyphi A. Res Microbiol. 2010;161(4):243–248. doi: 10.1016/j.resmic.2010.03.005

162.Achtman M, Wain J, Weill F-X, Nair S, Zhou Z, Sangal V, et al Multilocus Sequence Typing as a Replacement for Serotyping in Salmonella enterica. PLOS Pathogens. 2012;8(6):e1002776. doi: 10.1371/journal.ppat.1002776

163.Olsen SJ, Jim Pruckler, William Bibb, Nguyen Thi My Thanh, Tran My Trinh, Nguyen Thi Minh, et al Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol. 2004;42(5):1885–1889. doi: 10.1128/JCM.42.5.1885-1889.2004

164.Deborah House, John Wain, Vo A. Ho, To S. Diep, Nguyen T. Chinh, Phan V. Bay, et al. Serology of Typhoid Fever in an Area of Endemicity and Its Relevance to Diagnosis. J Clin Microbiol. 2001;39(3):1002–1007. doi: 10.1128/JCM.39.3.1002–1007.2001

165.Gopalakrishnan V, Sekhar WY, Soo EH, Vinsent RA, Devi S. Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella typhi*. Singapore Med J. 2002;43(7):354–358

166.Sattar AFMA, Yusuf MA, Islam MB, Jahan WA. Different Diagnostic Procedure of Typhoid Fever: A Review Update. J CurrAdv Med Res. 2014;1(2):35-41. doi: 10.3329/jcamr.v1i2.20517