

Review

Typhoid Fever Diagnosis in Endemic Countries: A Clog in the Wheel of Progress?

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Abstract: Typhoid fever causes significant morbidity and mortality in developing countries, with inaccurate estimates in some countries affected, especially those situated in Sub-Saharan Africa. Disease burden assessment is limited by lack of a high degree of sensitivity and specificity by many current rapid diagnostic tests. Some of the new technologies, such as PCR and proteomics, may also be useful but are difficult for low-resource settings to apply as point-of-care diagnostics. Weak laboratory surveillance systems may also contribute to the spread of multidrug resistant *Salmonella* serovar Typhi across endemic areas. In addition, most typhoid-endemic countries employ serological tests that have low sensitivity and specificity making diagnosis unreliable. Here we review currently available typhoid fever diagnostics, and advances in serodiagnosis of *S. Typhi*.

Keywords: Typhoid fever; *S. Typhi*; serodiagnosis; multidrug resistance; laboratory

1. Introduction

“Typhoid fever” (TF) was coined by the French physician, Pierre Charles Alexander Louis, who gave a description of the clinical signs and symptoms of the disease to be typhoidal, with signs of mental fogging and persistent fever which mimicked the symptoms caused by typhus [1,2]. TF is a systemic infection comprising of diseases caused by *Salmonella enterica* serovar Typhi; while *S. enterica* serovar Paratyphi (A, B and C) cause paratyphoid fever, other serovars of *Salmonella* are grouped as non-typhoidal. *S. Typhi* belongs to the family Enterobacteriaceae, which are Gram-negative rods and facultative anaerobes. The genus *Salmonella* is classified into two species, *S. enterica* and *S. bongori*, and more than 2600 serovars (or serotypes), based on its lipopolysaccharide (LPS) cell wall (somatic O antigen), the flagella (H antigen) and its surface Vi antigen (present only in *S. Typhi*, *S. paratyphi C*, *Citrobacter freundii*, and *S. dublin*) [3]. *S. Typhi* are restricted exclusively to human hosts and are associated with systemic infection, prolonged fever and may result in an asymptomatic carrier state. Following resolution of infection, a small number of persons, called carriers, continue to carry the bacteria. Around 2–5% of those who contract typhoid fever become chronic carriers, as the bacteria persists in the biliary tract after symptoms have resolved [4]. Seroepidemiological surveys have also revealed that some persons in the population have non-immunising antibodies present in them irrespective of previous vaccination status, and will be seropositive, which is a limitation of employing serodiagnostic rapid diagnostic tests (RDTs) in surveillance [5]. TF was an important cause of illness and death in the United States and Europe in the 19th century, as a result of overcrowded and unsanitary conditions. The provision of treated municipal water, pasteurization of dairy products and better sanitary conditions led to a steep decline in the incidence of typhoid in these regions [3,6]. In most developing countries, however, access to safe drinking water and adequate sanitation remains

a challenge. TF causes significant morbidity and mortality especially in Asia, Africa (although evidence suggests that the impact might be under-appreciated), Latin America and the Middle East. Despite the availability of vaccines, vaccination against TF remains limited in most of the affected countries. Lack of inclusion of typhoid vaccines into routine immunization programs in addition to poor TF surveillance and weak laboratory infrastructure in most countries affected further aggravates morbidity. TF is essentially a disease of the poor, uneducated and usually the most vulnerable in the society who lack access to basic social services such as health care, good hygiene and safe drinking water. Emergence of antibiotic resistance (ABR) strains of *S. Typhi* to fluoroquinolones has made management of TF in endemic countries even more challenging.

In most TF affected countries, physicians rely on laboratory results from serologic tests that are not reliable (usually Widal) to arrive at a clinical decision. Application of sensitive and specific diagnostic tests in health facilities, and establishment of active surveillance programs in endemic regions is important for accurate antibiotic prescriptions against local *S. Typhi* strains in circulation and detection of emerging ABR strains in the population. Here, we review laboratory diagnosis of TF, emergence of ABR *S. Typhi* and propose solutions to challenges encountered in TF-endemic countries.

2. Disease Burden

TF was estimated to cause approximately 21.65 million illnesses and 216,000 deaths worldwide in the year 2000 [7]. In 2010, the estimated number of cases after adjusting for water-related risk factors was 11.9 million cases and 129,000 deaths [7]. Studies report that TF is more prevalent in Asia; high-risk groups are children and infants, while those at risk of complicated TF include neonates and pregnant women [8,9]. In some countries in Asia, *Salmonella enterica* serovar Paratyphi A has accounted for a growing proportion of enteric fever; however, this is not the focus of this review [10].

3. Source and Mode of Transmission

Transmission of Typhoidal *Salmonella* is primarily through the fecal–oral route, i.e., through water or food contaminated with human feces. Contaminated water sources have also resulted in large epidemics of the disease [11,12]. TF is mainly found in developing countries, and it has been eradicated from developed countries through good hygiene, sanitary and availability of potable drinking water [13,14]. Poor hygiene practices by food handlers who usually lack good hygienic practices also encourages TF transmission in developing countries.

4. Pathogenesis

The minimum infectious dose of *S. Typhi* could be as low as 1000 or as high as a million bacteria based on studies carried out in human volunteers [15]. Following ingestion of the bacteria, it binds to mucosa cells in the small intestine, eventually invading the mucosa. Post invasion of the mucosa, the bacteria are translocated to the lymphoid follicles draining mesenteric lymph nodes, and some find their way into the reticuloendothelial cells of the liver and spleen. The bacteria are phagocytosed and have the ability to survive and multiply within macrophages of the lymphoid follicles, liver and spleen. Once the bacteria multiply to a certain threshold, in addition to their virulence and the host response, bacteria are released and sequestered into the blood stream. Following release into the bloodstream, they invade secondary sites such as the Peyer's patches of the terminal ileum, liver, spleen, bone marrow or the gall bladder [3]. Bacteria that are excreted in the bile re-invade the intestinal wall or are passed on through the feces. Cell counts of patients with acute TF gave a median bacteria concentration of 1 bacterium per ml of blood (about 66 percent of which are inside phagocytic cells) and about 10 bacteria per ml of bone marrow [16]. This indicates that the bone marrow has a higher concentration of bacteria and is a better site than blood for detection of *S. Typhi* in patients. *S. Typhi* infection has been shown to induce local and systemic immune responses in humans, but this provides incomplete protection against relapse and reinfection [17].

5. Laboratory Diagnosis of Typhoid Fever

Isolation of the causative bacteria in TF patients by culture remains the gold standard for diagnosis. Culture is the most reliable way of detecting typhoid in infected patients, and usually by blood culture, but bone marrow culture has a greater sensitivity. However, in most developing countries a serological test known as the Widal test is most commonly applied. Below we discuss the most commonly used laboratory approaches in typhoid detection in endemic countries and the challenges attributed to each (Table 1).

Table 1. Summary of available Typhoid Fever Diagnostic Techniques.

Diagnostic Tests	Principle	Sensitivity	Specificity	References
Non-immunodiagnostic Methods				
Blood Culture	Based on the ability of viable cell to grow on culture medium	15.38–51.8%	100%	[18,19]
Stool Culture		<50%	93%	[20,21]
PCR (without enrichment in blood culture)	Relies on amplification of gene of interest	90–100%	100%	[22–25]
Immunodiagnostic Methods				
TPTest	Measures <i>S. Typhi</i> membrane preparation (MP)-specific IgA responses in peripheral blood mononuclear cell culture secretions	96.0%	96.6%	[19,26,27]
Tube Widal	Measures agglutinating antibodies against O and H antigens of <i>Salmonella Typhi</i> and <i>Salmonella Paratyphi A</i> ; uses a tube or slide	65.38%	89.83%	[18,22]
Cromotest® O: semiquantitative slide agglutination		95.2%	3.6%	[21]
Cromotest® H: semiquantitative slide agglutination		80.3%	50.0	[21]
PanBio	ELISA detecting anti-LPS IgG and IgM	78%	80%	[28]
SD Bioline	ICT LFA cassette detecting IgG and IgM antibodies against an undefined <i>Salmonella Typhi</i> antigen	69%	79%	[29]
Mega <i>Salmonella</i>	ELISA detecting IgG and IgM antibodies against an undefined <i>Salmonella Typhi</i> antigen	91%	49%	[29]
LifeAssay Test-it	Detects IgM antibodies against <i>Salmonella typhi</i> LPS in an ICT LFA cassette	59%	98%	[30]
Typhidot	Measures IgM and IgG antibodies against a 50-kDa outer membrane protein of <i>Salmonella Typhi</i> in an immunodot test format	67–98%	58–100%	[29,31–34]
Typhidot M	Measures IgM antibodies, after removal of IgG antibodies, against a 50-kDa outer membrane protein of <i>Salmonella Typhi</i> in a dot blot format	47–98%	65–93%	[31,32,35]
TyphiRapid IgM and IgG IgM (Combo)	Measures IgM antibodies, after removal of IgG antibodies, against a 50-kDa outer membrane protein of <i>Salmonella Typhi</i> in an ICT LFAa cassette format	89–100%	85–89%	[36,37]
Tubex TF	Detects antibody against <i>Salmonella Typhi</i> LPS with an inhibition assay format and a visual result readout	56–100%	58–100%	[29,32–35,37,38]
Enterocheck-WB	Dipstick detecting anti-LPS IgM antibodies	89%	97%	[39]
Multi-Test-Dip-S-Ticks	Dipstick detecting anti-LPS IgG and IgM	89%	53%	[33]
PanBio	ELISA detecting anti-LPS IgG and IgM	78%	80%	[28]

6. Bacteria Culture

The definitive diagnosis of TF is isolation of *S. Typhi* from blood which should normally be sterile. Blood samples can be cultured on an enriched media (Blood agar) and a differential media (MacConkey agar). *S. Typhi* is a non-lactose fermenter producing smooth pale colonies on selective media. *S. Typhi* utilize citrate as carbon source, lysine as nitrogen source and produce hydrogen sulphide which is responsible for the characteristic black centers on *Salmonella-Shigella* agar or black butt with no gas on Kligler iron agar [40]. The volume of blood collected from patients is one of the key factors in

isolation of *S. Typhi*. Ideally 10–15 mL of blood should be collected from school children and adults, while toddlers and preschool children supply 2–4 mL of blood. Toddlers and preschool children require less blood because they have higher bacteremia than adults. The optimum ratio of blood to culture media for isolation of pathogens is 1:10. Blood specimens are inoculated into Brain heart infusion broth or tryptone soy broth immediately, incubated at 37 °C and subcultured at days 1, 2, 3 and 7 on blood agar (horse or sheep blood) or MacConkey agar and incubated at 37 °C for 18–24 h. Blood agar allows the growth of both Gram-positive and -negative pathogens, while MacConkey selects for Gram-negative pathogens only. In patients who have yet to initiate antibiotic treatment, within the first two weeks of infection, blood culture is positive in up to 80% of patients, while in patients already on antibiotics, sensitivity can be as low as 40% [41]. In patients already on antibiotics the preferred sample to be collected is the bone marrow, because there is a preponderance of bacteria at this site, where they multiply and reside. Blood samples for culture should be collected within 7–14 days, as bacteria counts in blood decline as disease progresses. The volume of blood collected is also a critical factor to consider in order to obtain positive results, because 1 mL of blood contains <10 bacteria [16,42–44]. In contrast, bone marrow culture is more sensitive giving good yields even when antibiotic treatment has been initiated. The reliability of bone marrow samples for culture is directly linked to a higher bacteria count in the sample. Isolation of the causative bacteria in culture makes it possible to carry out further testing such as antimicrobial susceptibility testing to determine the best antibiotic to be prescribed by the clinician, identification of multidrug resistant strains, epidemiologic typing and molecular characterization.

In resource-constrained settings, isolation of *S. Typhi* from stool/rectal swab culture which is more routinely used in most diagnostic laboratories is suggestive of typhoid fever. Stool samples should be collected in sterile wide-mouthed containers, and the quantity supplied by the patient directly affects the likelihood of isolation of *S. Typhi*. In the absence of stool samples, rectal swabs inoculated in Carry Blair transport medium (Thermo Scientific, Loughborough, UK) may suffice, though with limited success. Stool samples should be inoculated within 2 h of collection or stored at 4 °C until ready for inoculation. Approximately 1 g of stool sample is inoculated into 10 mL of Selenite F broth, 37 °C for 18–48 h. Following enrichment, subculture of Selenite F broth is made from the surface of the broth onto either MacConkey agar, Bismuth Sulfite agar, Deoxycholate Citrate agar, *Salmonella-Shigella* agar, Xylose-Lysine Deoxycholate agar or Hektoen Enteric agar, incubated at 37 °C for 24 h [45,46].

Some of the challenges in the application of culture-based approaches in isolating *S. Typhi* in endemic countries include the time taken to obtain culture results which takes at least 5–7 days, low sensitivity, lack of infrastructure and inadequate supply of trained manpower. In addition, another important limitation of culture method is the requirement of the use of antisera for confirmation of biochemical results indicating *S. Typhi*, which is resource-demanding for diagnostic laboratories in this setting.

DNA Detection

Development of molecular tests for TF diagnosis requires genetic markers that are sensitive and specific for detection of bacterial DNA in blood of febrile patients [47]. Nucleic acid amplification tests, including conventional PCR, nested, multiplex and real-time PCR, have been developed for the detection of *S. Typhi* in blood [16,23,25,48–51]. 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. Application of molecular techniques in clinical settings has technical limitations because of the few number of bacteria in blood, approximately 0.5 CFU/mL [16,51]. Molecular tests usually include primers targeting *fliC-d* in order to provide good specificity with little overlap with environmental isolates. Molecular detection of *S. Typhi* DNA in human blood has also been optimized through removal of background human DNA in order to improve the sensitivity of PCR for bacterial DNA and also reduce false positives. Enriching the PCR protocol to select for target bacterial DNA has been demonstrated to improve sensitivity by

at least 1000-fold, compared to conventional methods of DNA preparation [52]. In addition, some researchers have further optimized molecular detection of *S. Typhi* DNA in human blood by inclusion of an incubation step. This technique described as blood culture-PCR, which includes a brief incubation step prior to PCR, has been developed in order to improve sensitivity and specificity of the assay. Blood samples of 5 mL were collected from controlled human infection models and incubated in ox-bile for 5 h, DNA extracted and amplified with primer targeted at *S. Typhi* fliC-d. This optimized molecular approach enabled the detection and confirmation of typhoid infection cases that would have been missed by blood culture [53]. Some of the limitations of molecular techniques include the identification of non-febrile patients with DNA in blood or bacteria shedding in stools leading to false positives. The main challenge with development of molecular assays is the applicability of these assays in resource-poor settings.

7. Serologic Testing

7.1. Widal Test

The Widal test was developed by Georges Ferdinand Widal in 1896 and helps to detect the presence of *Salmonella* antibodies in a patient's serum. The Widal test measures agglutinating antibodies against the O and H antigens of *S. Typhi* in sera of people with suspected TF. Patients infected with *Salmonella* produce antibodies against the antigens of the organism. Antibodies in serum, produced in response to exposure to *Salmonella* antigens, will agglutinate bacterial suspension which carries homologous antigens. Antigens prepared from *Salmonella* are mixed with the patient's serum to detect the presence of the antibodies. Agglutination reaction suggests a positive result, while absence suggests a negative result. In principle, to carry out the Widal test acute- and convalescent-phase serum samples should be collected approximately 10 days apart; a positive result is determined by a 4-fold increase in antibody titer [54]. However, antibody titers in infected patients often rise before the clinical onset of disease; this makes it challenging to demonstrate a 4-fold increase. In addition, in cases where patients supply paired sera, a decrease in titer is commonly observed when comparing the convalescent-phase serum to acute-phase serum. This might be due to the fact that most patients visit clinics during the convalescent phase, after initial pretreatments have failed.

The Widal test is simple and inexpensive to perform, and widely used in developing countries, although it has limited diagnostic value. However, it suffers from a lack of standardization of reagents, poor specificity and inappropriate results interpretation [55]. Also, in areas of endemicity there is often a low background level of antibodies in the normal population, this makes it difficult to determine the appropriate cut-off point for a positive result due to differences between areas and between times [22]. Proper interpretation of Widal test results requires that each country determines the appropriate titer with which to diagnose typhoid.

7.2. Other Rapid Serologic Tests (Non-Widal)

Test characteristics of some commercially available RDTs are summarized in Table 1, but none of the RDTs have high sensitivity and/or specificity needed. Lack of specificity and sensitivity of Cromotest[®]—semiquantitative Slide agglutination, Tubex[®], Cromotest[®]—single-tube Widal and Typhidot[®] have been demonstrated elsewhere [21]. Other limitations of RDTs include limited application, difficulty in interpretation and affordability [56]. In the Philippines, the cost of a single test was as high as \$51.68 for Tubex and \$23.52 for Typhidot in some hospitals [29]. When compared with the Widal test which may not cost more than \$1.5 per sample, these RDTs are not cost-effective and not affordable, especially in low-resource settings.

Tubex test, a colorimetric assay, might also pose difficulty in interpretation of hemolyzed samples. In addition, there might be false positives in patients infected with *S. enterica* serotype Enteritidis since Tubex detects immunoglobulin M directed towards *S. Typhi* O9 lipopolysaccharide antigen in sera. Other serological assays for TF that have been commercially developed include the

Counter-current Immuno electrophoresis test, which 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, but bands are often difficult to see and it is more expensive [57]. Additionally, several urine assays have been developed for TF testing although none has proved effective in detecting TF, despite the advantages of carrying out urine testing in resource-poor settings that are endemic for TF [58]. Generally, although RDTs have demonstrated some improvement over the Widal test, they still lack the required sensitivity, specificity, cost-effectiveness and consistency to allow their use as point-of-care diagnostics in endemic settings [59].

Available RDTs have variations in target antigens, for example, Typhidot detects specific IgM and IgG antibodies against the 50 kDa outer membrane protein (OMP). There is a modification of the Typhidot called Typhidot-M, detecting only IgM produced against the OMP, which is specific in acute infection. Additionally, because it detects only recent infections, it narrows the possibility of false-positive result due to previous infection. Multi-Test Dip-S-Ticks which detects five antibodies; the SD Bioline Typhoid rapid test, which uses an immunochromatographic method to detect IgG and IgM antibodies against an undefined *Salmonella* serovar typhi antigen; and a dipstick test named Enterocheck-WB that detects anti-LPS IgM antibodies [22,29,33]. SD Bioline, therefore, offers the advantages of detecting both chronic and acute infections, it is rapid (15–il min), and serum, plasma or even whole blood can be analyzed [59]. However, since detecting single antibody with single-antigen target have been demonstrated to increase specificity, multi-antibody approach may decrease specificity.

The abundance of IgG in the sera of people in highly endemic settings could limit the use of RDTs in these settings. For example, the use of Typhidot in highly endemic settings is limited by the abundance of IgG which can persist for more than 2 years [60], hence detection of specific IgG cannot differentiate between acute and convalescent cases. In order to mitigate the problem of sensitivity, an IgG depletion step was applied which led to the development of Typhidot-M which removed competitive binding and allowed accessibility of the antigen to the specific IgM [61].

7.3. Novel Biomarkers for Serodiagnosis

Serological markers currently available for typhoid diagnosis have a low degree of sensitivity and specificity. This necessitates the discovery of new targets that could be employed in serological analysis in the laboratory for accurate results. ELISAs have been developed and tested in detecting *S. Typhi* in endemic countries. A recent study in Nigeria tested IgA, IgM and IgG ELISAs using *S. Typhi* LPS and hemolysin E (HlyE) proteins on children with acute TF. The candidates for the ELISAs in the Nigerian setting was based on a proteome microarray data previously carried out by the same research group where they identified hemolysin E and LPS as putative biomarker targets. The receiver operator characteristic area under the curve (ROC-AUC) values suggested that LPS-specific IgA and IgA+M ELISA, in particular, was sensitive in diagnosing acute typhoid, and could discriminate well between typhoid and healthy, and other febrile bacteremias commonly encountered in Nigeria [62,63]. In another study in Bangladesh, 12 proteins were expressed and purified to design ELISAs and tested in a cohort of febrile patients. ELISAs were designed to detect IgM-specific antibodies to the 12 purified protein antigens and *S. Typhi* Vi polysaccharide antigen. Further analysis of ROC-AUC values revealed that the best three candidate antigens for serodiagnosics were encoded by STY4539 and STY1886 in combination with the Vi polysaccharide [64].

In a recent study, 4445 *S. Typhi* antigens were used to probe sera of individuals challenged with *S. Typhi* Quail strains. Humoral immune responses were measured throughout duration of illness in the human challenge models. The study identified putative serodiagnostic biomarkers which include components of the bacterial cell surface (OmpA) and proteins targeted toward host cell attack (HlyE) and invasion (SipC) [65]. Other *S. Typhi* proteome array studies have indicated HlyE as a useful serodiagnostic marker based on IgA and IgG responses [62,65–67]. OmpA was also identified as a useful biomarker from proteomic screening [67] but is limited by being cross-reactive, expressed by

other *Salmonella* and might not be a useful discriminator of *S. Typhi* infection in resource-poor settings where exposure to other *Salmonella* antigens might be frequent. *S. Typhi* proteome array screening has also identified two new putative biomarkers; *N*-acetylmuramoyl-L-alanine amidase (t2002, STY0927), which is involved in the catabolism of peptidoglycans and has previously been associated with invasion and intracellular survival of *Salmonella* Typhimurium and an uncharacterized hypothetical protein, t2295. Both putative protein targets gave IgM responses in controlled human infection models [64]. Inclusion of LPS into biomarker panels for *S. Typhi* screening seems to give better serodiagnostic value than panels without LPS as suggested in a study among pediatric children in Nigeria and in Nepal [62,65]. Antibody in Lymphocyte Supernatant assay (ALS) which has been used during vaccination and in other infections such as cholera [68], tuberculosis [69] and influenza [70] has also been applied to identify anti-*S. Typhi* antibodies in infected cohorts in Bangladesh [26]. More recently, ALS is being applied to decipher new serodiagnostic markers through immunoprofiling studies to identify immunodominant antigens that could be used in development of rapid serodiagnostics [67,71]. In all, recent *S. Typhi* proteome array studies suggest the need for inclusion of immunoglobulin targets specific to some of the putative proteins that have yielded better diagnostic value for evaluation in endemic settings. Some of the putative protein targets that should be considered for future diagnostic development for RDTs based on data from endemic countries are an IgA to *S. Typhi* LPS and IgG to HlyE for better diagnostic results in resource-limited settings [71]. These two targets have provided promising evidence of their usefulness as novel biomarkers for serodiagnosis in resource-poor settings endemic for *S. Typhi* infections.

8. Challenges in the Diagnosis of TF in Endemic Countries

TF is endemic in countries that are classified by the World Bank as Low-income or Low-middle-income countries (LMIC), or commonly referred to as developing countries. These countries are also plagued by other diseases such as malaria, tuberculosis and HIV, which compete for attention from the countries' health sectors, in addition to several neglected tropical diseases. Poor laboratory infrastructure is a major impediment in TF-endemic countries. For example, in TF-endemic countries in Africa, most laboratories do not have the facilities and laboratory support to carry out blood culture, and the technical needs of bone marrow culture would make it very unlikely to be successfully performed in most of these settings. This implies that laboratories in resource-poor settings would usually rely on the poor diagnostic sensitivity of the Widal test for physicians to make clinical decisions on TF. In addition, there is no active surveillance system in place to get the actual estimates of disease burden, since most data available on TF are based on those that present to the hospital for treatment which would be a fraction of the actual number of cases, since pregnant women, neonates and those presenting to other departments in the hospital are not screened for TF. Under-estimation of the true disease burden has led to poor focus on TF, and reduced resource allocation in most affected countries [9]. Lack of rapid diagnostic tests that are affordable, very sensitive and specific has also hampered progress in TF diagnosis and treatment. Specimen collection remains an issue in TF diagnosis, as some of the laboratories do not have the manpower and infrastructure for timely collection and processing of blood samples in suspected patients. Over-diagnosis of TF in resource-limited settings owing to misuse of the Widal test has also resulted in the indiscriminate prescription of antibiotics. Furthermore, inappropriate antibiotic use due to their availability over the counter without isolation of the bacteria *S. Typhi* has contributed to the spread of Multidrug Resistant (MDR) *S. Typhi*. The emergence and spread of MDR *Salmonella* strains and decreasing efficacy of anti-typhoid drugs in endemic countries are huge sources of concern to public health stakeholders [72]. Chloramphenicol, Trimethoprim-sulphamethoxazole and ampicillin were the drugs of choice for many years for the treatment of typhoid fever, but are no longer in use in many countries because of the emergence of plasmid-mediated resistance about three decades ago [72,73]. To ensure better therapeutic results, fluoroquinolones, such as ciprofloxacin and ofloxacin, are still drugs of choice in endemic countries [57] instead of ceftriaxone due to their availability and affordability, even after the patent

on these drugs expired in 2003 [73]. Unfortunately, strains of *S. Typhi* resistant to fluoroquinolones have emerged, causing increased treatment failure, disease severity, and even death [74,75]. Attempts to use vaccination in control of TF has also not been 100% effective. Vaccination targeting infants may not be sufficient to control the disease, since the epidemiology of TF suggests that a significant disease burden occurs in older populations beyond infancy and early childhood [76]. Three types of *Salmonella* vaccines are currently licensed; the oral live attenuated *S. Typhi* Ty21a vaccine, injectable Vi polysaccharide vaccine, and the recently approved Vi-tetanus toxoid (Vi-TT) conjugate vaccine [77,78]. However, these vaccines do not confer complete protection when administered, thus transmission and outbreaks are not fully preventable.

9. Conclusions

Without improved diagnostics, accurate estimates of the burden of typhoid, and planning by technical partners and governments concerned will remain challenging. There is a need for increased funding for laboratory infrastructure in developing countries especially in Sub-Saharan Africa where the true burden of typhoid is underappreciated, largely due to weak public health systems. While these efforts are being put in place, public health authorities in endemic countries should establish a threshold that would be used to determine positive Widal tests for proper interpretation of the most readily available, easy-to-perform and relatively noninvasive and affordable diagnostic, Widal in resource-poor settings. In addition, there should be a local surveillance data of enteric infections and other illnesses that cross-react with *Salmonellae* antigens in order to guide clinicians in interpretation of Widal test results. The approval by World Health Organization of the new Tybar-TCV[®] vaccine suitable for children as young as 6 months, requiring a single dose with at least 55% protection, presents an opportunity for countries affected in Asia and Africa to incorporate TF vaccination into their routine childhood immunisation as an approach to reduce disease burden [79]. Lastly, there is an urgent need for rapid and improved diagnostics for accurate surveillance and monitoring impact of control efforts.

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