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Enteric Fever: Diagnostic Challenges and the Importance of Early Intervention

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Abstract

Enteric fever is a systemic infection caused by highly virulent *Salmonella enterica* serovars: Typhi and Paratyphi. Diagnosis of enteric fever is challenging due to a wide variety of clinical features which overlap with other febrile illnesses.

The current diagnostic methods are limited because of the suboptimal sensitivity of conventional tests like blood culture in detecting organisms and the invasive nature of bone marrow culture. It emphasizes the need to develop improved and more reliable diagnostic modalities. The rising rates of multidrug-resistant *Salmonella* strains call for an accurate understanding of the current management of the disease. Proper public health measures and large-scale immunization programs will help reduce the burden of the disease. A comprehensive surveillance system can help detect the chronic carrier state and is crucial in understanding antibiotic susceptibility patterns.

We conducted an all-language literature search on Medline, Cochrane, Embase, and Google Scholar till May 2022. The following search words and medical subject headings (MeSH) were used: "enteric fever," "*Salmonella* Typhi," "multidrug-resistant *Salmonella*," chronic carrier state," "*Salmonella* detection, "and "typhoid vaccine." We reviewed the literature on clinical features, pathophysiology, new diagnostic tests, and interventions to prevent the disease.

This article explores enteric fever and its various clinical features and addresses the emerging threat of multidrug resistance. It focuses on novel methods for diagnosis and prevention strategies, including vaccines and the use of surveillance systems employed across different parts of the world.

Categories: Internal Medicine, Pediatrics, Infectious Disease
Keywords: surveillance, typhoid vaccines, serological tests, chronic carrier, multidrug resistant salmonella

Introduction And Background

Enteric fever is a systemic infection caused by *Salmonella enterica* (*S. enterica*) serotypes, Typhi (most common) and Paratyphi A, B, and C. It attributes to significant morbidity and mortality in resource-limited countries like Asia, Africa, and South America with a limited potable water supply and improper sanitation [1]. According to WHO, annually 21 million enteric fever cases and up to 161,000 deaths are reported worldwide [1]. However, a recent study about the global burden of typhoid and paratyphoid fever in 2017 reported a 41% decline in deaths from enteric fever compared to 1990 [2].

Transmission of *Salmonella* Typhi (*S.* Typhi) and *Salmonella* Paratyphi (*S.* Typhi) occurs fecal-orally from contaminated food or water. There is a short-cycle transmission in which the bacteria contaminate the environment from acute or chronic carriers due to inadequate sanitation, while pollution of big water bodies by sewage is a long-cycle transmission [3].

Signs and symptoms of enteric fever are wide-ranging and mimic other systemic illnesses. It includes fever, chills, headaches, anorexia, abdominal discomfort, relative bradycardia, vomiting, diarrhea, constipation, hepatomegaly, splenomegaly, leukopenia, and thrombocytopenia. The clinical features and complications can also vary according to age, making a clinical diagnosis of enteric fever difficult [4,5]. Common complications include anemia, gastrointestinal bleeding, intestinal perforation, bone marrow hypoplasia, encephalopathy, disseminated intravascular coagulation, and shock. The reports showed that the 10-30% case fatality rate drops to 1-4% with treatment [4]. A study reviewing 83 reports of enteric fever cases from South Asian countries showed that preschool children (6%) had the highest case fatality rates compared to

other age groups [5]. Hence, it is imperative to diagnose enteric fever early to prevent mortality. Furthermore, 2-5% of patients, even after recovery, become asymptomatic carriers and continue to shed the bacteria [6,7].

The current case definition of enteric fever is fever ⩾38 °C for at least three days with a positive culture of bacteria from blood/bone marrow. However, several factors are limiting the definitive diagnosis: the volume of blood required (usually more than 7 mL), depending on the age of the subjects due to low levels of bacteremia (we also need to transport blood at ambient temperature (15-40˚c)); low sensitivity ~50% for blood culture and ~80% for bone marrow culture, even though the specificity reaches 100% for both; and lack of resources, including trained personnel and laboratory equipment.

According to WHO, the gold-standard diagnosis of typhoid fever should approach 100%, each for sensitivity, specificity, and positive and negative predictive values [1,8].

The recommended antimicrobial treatment for enteric fever includes chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, third-generation cephalosporins, and azithromycin. The recent emergence of multidrug-resistant (MDR) *Salmonella* has limited the use of many of these drugs. Many mechanisms, such as acquiring chromosomal mutations by a transposon and plasmid exchange cause the resistance, and haplotype-58(H58) is the most dominant strain [1,8].

There are two vaccines licensed and accessible for enteric fever which is summarized in Table *1*. The choice of a vaccine depends on the target age group, vaccine efficacy, patient compliance, and doctor's preference.

Vaccine	Route	Number of doses	Age for administration	Type of vaccine
Vi capsular polysaccharide vaccine (Vi-Ps)	Injectable	Single dose	Above two years	Subunit antigen
Ty21a strain vaccine	Oral	Three doses	Above five years	Live attenuated

TABLE 1: Types of typhoid vaccines

A study in Chile reported 67% of accumulative efficacy after three doses of oral vaccine (one capsule every other day) at three years and 62% at seven years of follow-up [9,10].

In conjunction with routine immunizations, interventions like improving water quality and sanitation and supervision of antimicrobial resistance followed by reporting the antimicrobial susceptibility data can help in mitigating the mortality and morbidity burden of enteric fever worldwide [11].

Review

Pathophysiology

Enteric fever, one of the foremost bacterial infections worldwide, is mainly caused by *S. enterica* serovar Typhi [12]. *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B, and Paratyphi C are called typhoidal *Salmonella* serovars because Paratyphi strains cause similar clinical symptoms [13,14]. *S.* Typhi and Paratyphi are pathogens restricted to humans and not common in other *Salmonella* serovars. *S.* Typhi exists as a Gram-negative encapsulated, flagellated, facultative anaerobic bacilli. It possesses three major antigens: H or flagellar antigen, O or somatic antigen, and Vi antigen [15].

The infection transmits through poor hygiene and the fecal pollution of food and water [16]. The incubation period is mostly 7-14 days. After establishing an initial, clinically undetectable infection and transient primary bacteremia, the organism disseminates systemically to the liver, spleen, bone marrow, Peyer’s patches of the terminal ileum, and gallbladder [17,18]. Large numbers of bacteria then spill into the bloodstream, initiate secondary bacteremia, and manifest definite clinical symptoms.

Typhoid induces mucosal, humoral, and cellular immune responses, but these do not grant complete protection in reinfection and relapse [19]. Specialized epithelial M cells in Peyer’s patches are the potential site of invasion of *Salmonella* by a pathologically distinct process, unlike the conventional receptor-mediated endocytosis [20]. The pathogenesis of typhoid is illustrated in Figure *1* [21].

FIGURE 1: Pathogenesis of typhoid

The pathological hallmark of enteric fever is mononuclear cell infiltration and hypertrophy of the reticuloendothelial system, including the intestinal Peyer's patches and mesenteric lymph nodes [22]. Recent data showed another alternative method used by *Salmonella* to disseminate from the gastrointestinal tract by passively traversing the epithelial barrier using CD18-positive phagocytes [23].

Salmonella Pathogenicity Islands

Different pathogenic *Salmonella* strains have evolved by obtaining a group of virulence (Vi) genes in a contained area of the chromosome called *Salmonella* pathogenicity islands (SPIs) [24]. Diverse SPIs of variable sizes showed that they encrypt many Vi factors that help the organism survive, adhere, invade, and produce toxins.

S. Typhi encodes four relatively distinct SPIs: SPI-7, 8, 15, and 18, and these SPIs have a significant contribution to Vi. SPI-7 encodes Vi capsule, type IV B pilus, and via B locus [25]. The Vi capsule holds vital immunomodulatory functions such as dampening immune response, escaping peroxide-mediated killing, and preventing complement activation [26,27]. SPI-11- encodes typhoid toxin.

Type III Secretion System

Several pathogenicity islands, including SPI-1 and SPI-2, encrypt specialized tools to inject Vi proteins into host cells, termed type III secretion system (TTSS), which are distinct Vi phenotypes of each pathogen [28]. These injected bacterial components change fundamental host-cell functions like signal transduction, membrane trafficking, and cytoskeletal arrangement. Extracellularly, the SPI-1 TTSS helps to attack non-phagocytic cells and induce intestinal inflammatory responses [29,30]. On the contrary, the SPI-2 TTSS comes into action after internalization and helps to promote the development of the *Salmonella*-containing vacuole (SCV) and intracellular replication [31]. Amusingly, several *Salmonella* TTSSs genes are present within temperate bacteriophages, which indicates that horizontal gene transfer allows fine-tuning of Vi phenotypes by mixing different TTSS effector proteins [32,33].

Pathogen-Associated Molecular Patterns

Salmonella strains express distinct structures on their surface, such as Toll-like receptors (TLR) and NOD-like receptors. They are pathogen-associated molecular patterns (PAMPs) [34]. Naturally, host innate immunity can identify PAMPs; however, *S.* Typhi Vi capsular polysaccharide and the typhoid toxin do not trigger a pro-inflammatory response by hindering the PAMPs and, thereby, allowing them to withstand intracellular development in the reticuloendothelial system [35]. After invading the intestinal cells, macrophages in the lamina propria overtake the bacteria, and regardless of bactericidal activities in host cells, *S.* Typhi can persist and replicate in macrophages using SPI-2. *S.* Typhi also triggers via B locus expression and silences flagella expression to prevent TLR recognition and successive IL-8 production [36,37]. Thus, one of the mechanisms by which *S.* Typhi escapes the adaptive immune system is lowering TLR signaling, which halts the induction of the IL-12/IFN-γ axis of lymphocytes.

Typhoid Toxin

Another Vi factor linked with typhoidal *Salmonella* is an atypical AB toxin, secreted within vesicles originating from SCV and released into the extracellular space, where it binds to Neu5Ac-terminated receptors on target cells inducing G2/M cell cycle arrest and cell death [38,39]. Following export, typhoid toxin infects various cells through autocrine and paracrine pathways [39]. *S.* Paratyphi A lacks Vi capsule, but they can intricate long O-chains, which helps to avoid antibody-mediated complement activation [40].

The non-typhoidal *Salmonella* serovars (NTS) (e.g., *S.* Enteritidis and *S.* Typhimurium), which primarily cause gastroenteritis also infect the same route. However, their clinical and pathological courses are quite different. While NTS gastroenteritis has a massive neutrophil influx which keeps the infection localized and restricts it to a short course of symptoms, enteric fever does have a long course of illness with delayed onset. Additionally, the invasion of macrophage-like cells with *S.* Typhi results in noticeably low production of the neutrophil chemoattractant interleukin (IL)-8 compared to invasion with *S.* Typhimurium [41]. These findings suggest that *S.* Typhi holds unique Vi traits that allow it to down-regulate pattern recognition receptors (PRRs) mediated host response in the intestinal mucosa [42].

Threatening the emergence of multidrug resistance strains

A major revolution in the treatment of enteric fever was the introduction of chloramphenicol in 1948. Ampicillin and trimethoprim/sulfamethoxazole were used as alternatives, whereas chloramphenicol use was not applicable [43]. However, chloramphenicol was used widely and often erratically due to its orally available form, broad-spectrum range, and cheap rate resulting in the blooming of resistance.

The efficacy of chloramphenicol remained pleasing until 1989; however, there was a quick emergence of MDR *S.* Typhi (resistant to three first-line antibiotics recommended by the WHO: chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) in several parts of India [44]. A study conducted across typhoid-endemic Asian countries reported that the prevalence of MDR *S.* Typhi strains climbed from 7% to 65% [45]. Studies show IncHI1 plasmids embrace various genes on composite transposons in the MDR strains approaching high transmissibility [46,47]. Possession of the R plasmid encoding acetyltransferase enzyme is the reason for chloramphenicol inactivation, which results in resistance.

The genes responsible for ampicillin and trimethoprim-sulfamethoxazole resistance are TEM-1 β-lactamase and dihydrofolate reductase type VII. MDR enteric fever is more dangerous because its complication and mortality rate is higher than sensitive strains. High mortality rates may be related to the increased Vi of multidrug-resistant *Salmonella* and a higher number of circulating bacteria [48].

Resistance to older antibiotics has forced researchers to develop the next best option to combat salmonellosis, including fluoroquinolones (mainly ciprofloxacin) and extended-spectrum cephalosporins [49,50]. Fluoroquinolones aim for DNA gyrase and topoisomerase IV, the bacterial enzymes playing a major role in their DNA transcription. For moxifloxacin and gatifloxacin resistance, the primary target of bacteria is the gyrA gene mutation (which encodes DNA gyrase). For levofloxacin and ciprofloxacin, it is the parC gene (encodes topoisomerase IV) [51,52]. Whole-genome sequence analysis of diverse *S.* Typhi isolates revealed the spread of a single *S.* Typhi haplotype, termed H58 (or genotype 4.3.1.), which is highly responsible for multidrug resistance and reduced fluoroquinolone susceptibility [53,54]. A steady increase noticed in the minimum inhibitory concentration (MIC) of fluoroquinolones was due to the rapid emergence of the H58 S. Typhi genotype in South Asia. Currently, it is no longer considered a first-line typhoid treatment [55-57].

In reply to the rise of fluoroquinolone resistance, third-generation cephalosporins have been a rescuer in treating enteric fever [58]. Still now, ceftriaxone and cefotaxime have remained trustworthy antimicrobial resources specifically for inpatient cases [59]. While the term MDR described resistance to three first-line antibiotics, extensive drug resistance (XDR) exhibited resistance to fluoroquinolones and third-generation cephalosporins in addition to first-line antibiotics [44]. *S.* Typhi holds the capability to alter from MDR to XDR by cherishing an IncY plasmid, which also confers resistance to fluoroquinolones. In addition, horizontal acquisition of this plasmid produces extended-spectrum beta-lactamases (ESBLs) through the blaCTX-M-15 gene can result in cephalosporin resistance [60]. Indiscriminate and offhand use of these cephalosporins led to the advancement of varied ESBL types such as PER, SHV, and TEM enzymes [61,62]. Reports of a high sporadic ceftriaxone resistance validate this statement [25]. The advent of ESBLs in MDR strains indicates urgent reconsideration of the use of cephalosporins, especially in developing countries [63].

If the MDR strains have intermediate susceptibility or resistance to ciprofloxacin, oral Azithromycin, a broad-spectrum azalide, is an effective alternative. It is very effective in removing intracellular *Salmonella*. Other beneficiary properties include low relapse rate, rapid defervescence, convalescent fecal carriage, favorable outpatient compliance, and pediatric suitability [64,65]. Although currently rare, *S.* Typhi strains with high azithromycin MICs and treatment failure have been stated [66]. Azithromycin resistance is due to the msrA, msrD, and ereA genes [53]. Nevertheless, if we encounter XDR strains during clinical practice, the treatment options become extremely limited to expensive drugs such as tigecycline or carbapenems (e.g., meropenem, imipenem, and ertapenem). However, minimal studies are currently present to show their efficacy in the treatment of enteric fever. This necessitates systematic, large-scale studies to assess the relative advantages of these drugs in treating enteric fever [67].

Antibiotic resistance has emerged as a massive challenge in the management of enteric fever, and threats to the growth of *S.* Typhi resistance consist of irrational prescription of antimicrobial combinations, easy accessibility of drugs at the pharmacy, unrestrained use of antibiotics in agriculture, and failure to trace chronic carriers [14,68].

The ideal management of enteric fever emphasizes the local pattern of antimicrobial resistance and antimicrobial susceptibility testing of the isolates [46]. Some forthcoming approaches to alleviate drug resistance in developing countries include judicious antibiotic prescribing practices by healthcare personnel and vaccination of at-risk people in endemic zones [48]. Interestingly, the re-emergence of chloramphenicol and co-trimoxazole vulnerable strains has shown the path for reintroducing the first-line antibiotics, but there are questionable concerns regarding their relapse [69,70]. Combination therapy might improve antibiotic-resistant states by synergistic action, but we have limited proof to validate this approach [71]. Moreover, as XDR strains flare up, novel therapeutic approaches like bacteriophage therapy will be needed to resist the infection [72,73].

Clinical manifestations, complications, and hostile chronic carrier state

Enteric fever is a common bacterial infection with nonspecific findings [1]. High-grade continuous fever (step ladder pattern) is the most essential and common presenting feature in the initial stage. The incubation period is 7-14 days as mentioned above. Patients may have nonspecific findings such as chills, abdominal pain, headache, constipation or diarrhea, weakness, dizziness, nausea, and cough. The severity of symptoms can fluctuate from being able to maintain normal activities of daily living to patients requiring inpatient care [74].

The patient may have diffuse abdominal tenderness, hepatosplenomegaly, and lymphadenopathy on physical examination. Rose spots, which appear during the second week of illness, are fine diffuse maculopapular rashes seen on the trunk and spreading to arms and legs. They are pathognomonic findings of enteric fever and are present in 3% to 40% of the patient. Some patients may also have relative bradycardia [75].

Feces represent the primary portal of exit of *S.* Typhi, although shedding in urine has also been documented [76]. Surveillance of patients with acute typhoid fever has demonstrated that up to 10% of the untreated patients excrete *S.* Typhi in their stools for up to three weeks following infection and are called chronic carriers. Carriers of *S.* Typhi can excrete between 10^6 and 1010 organisms per gram of feces, a bacterial load that is large enough to promote fecal-oral transmission of the organism. People who work as food handlers comprise most of them [75].

Untreated acute enteric fever may result in complications (21-34%) irrespective of age. Patients may present with gastrointestinal tract complications, hematological, cardiovascular, pulmonary, and other organ systems.

Table *2* lists all the untreated or progressive infection complications according to the type of organ system involved [77].

Digestive system	**Hepatitis, liver abscess, cholecystitis (predominantly acalculous type), paralytic ileus, ileal ulcer and gastrointestinal bleeding, intestinal perforation, pancreatitis**
Blood and lymphatic system	Anemia, leukopenia, thrombocytopenia, bone marrow suppression (resulting in pancytopenia), disseminated intravascular coagulation, hemophagocytosis, splenic infarction, and abscess
Cardiovascular system	Myocarditis, sick sinus syndrome, and heart block
Central nervous system	Psychosis, and encephalopathy
Respiratory system	Acute respiratory distress syndrome, pneumonia, pleural effusion, bronchitis, and lung abscess
Musculoskeletal system	Osteomyelitis (in children with sickle cell disease) and arthritis/arthralgia
Endocrine	Syndrome of inappropriate ADH secretion
Miscellaneous	Soft tissue abscess and mycotic aneurysms

TABLE 2: Complications of enteric fever

ADH: antidiuretic hormone

Enteric fever leads to hypertrophy of the reticuloendothelial system, but necrosis and ulceration are mostly limited to Peyer's patches distribution. The lethal complications of typhoid fever are intestinal hemorrhage and perforation. Perforation is typically located in the region of the terminal ileum and occurs secondary to necrosis of Peyer's patches. The typhoid intestinal perforation reported incidence is between 0.8% and 18% [78].

Diagnostic challenges, available options, and novel approaches

Low socioeconomic countries account for the highest burden of enteric fever, with more than 21 million episodes per year. However, this can be misleading due to the lack of accurate tools for diagnosis [79,80]. They prefer a clinical diagnosis that is often erroneous over diagnostic tests in this context. Nevertheless,

even making a clinical diagnosis is a challenge because clinical manifestations such as elevated temperature and white blood cells, low platelets, fatigue, and anorexia are not exclusive to enteric fever [81,82]. Identical symptoms can occur in diseases like dengue virus, influenza virus, tuberculosis, malaria, typhus, or even leishmaniasis, which are endemic in many developing countries [83].

Conventional Salmonella Detection

The gold-standard diagnostic method for enteric fever is the blood or bone marrow culture. Fever more than 38°C for at least 72 hours in addition to positive blood or bone marrow culture is the definitive diagnosis of enteric fever [50]. Both blood and bone marrow culture have a 100% specificity and allow profiling of the antibiotic resistance of the organism [50]. However, it requires technical skills, expertise, and facility. It is not ideal in endemic areas where results are required rapidly. The sensitivity of blood culture is about 50%, while that of bone marrow is about 80% [50]. The wide-scale application of the bone marrow culture is not feasible because of its invasive nature.

Serological Detection

The serology tests for enteric fever will only suggest the presence of the infection. It is easy, provides results rapidly, and we use it mainly in endemic areas. A serological test for enteric fever identifies the presence of antigens, mainly the Vi, lipopolysaccharide O, and flagellar H antigens.

These antigens are also present in other *Salmonella* serovars; therefore, specificity is not 100%. The Vi antigen is positive in *S.* Typhi and *S.* Paratyphi C and negative in *S.* Paratyphi A and B [84]. It is helpful as a screening test for chronic carriers [85].

A constraint in the Vi antigen serology-based test is the recent identification of some *S.* Typhi that are Vi negative and certain conditions that must be present before the Vi-positive *S.* Typhi can express the antigen [86,87].

The a, b, c, and d are flagellar H antigens present in *S.* Paratyphi A, *S.* Paratyphi B, *S.* Paratyphi C, and *S.* Typhi, respectively. Other flagellar H types present on *S.* Typhi are the j and z66 [1,88].

Widal Test

Agglutination reaction between the flagellar H antigen, lipopolysaccharide O antigen, and antibodies specific to them in the serum is the basis of the widely used Widal test [89,90]. Ideally, the Widal test is positive when done twice with a difference in antibodies level of about fourfold. However, it is generally done once due to low resources in developing countries where enteric fever is endemic [91-93].

Rapid Diagnostic Tests

Newer rapid diagnostic methods that are also cheap and show promising indicators include the Typhidot and Tubex TF, produced in Malaysia and Sweden. SD Bioline, Mega *Salmonella*, and Typhidot M are the new versions, but there is little information regarding their reliability [94-97].

Typhidot uses the enzyme-linked immunosorbent assay (ELISA) technique, which allows IgG and IgM against the 50kd outer membrane protein of the *S.* Typhi present in the serum of the patient. On the other hand, Tubex TF utilizes the reaction between the monoclonal antibodies, which are bound to the 09 lipopolysaccharides of the *S.* Typhi, and IgM antibodies present in the patient's serum.

Biomarkers

A promising method of diagnosis involves investigating a series of new biomarkers by seeking the different unique biomarkers found in the plasma of patients with acute enteric fever. These methods of diagnosis show increased sensitivity and specificity [98-101]. They can distinguish acute infection from previous infection and vaccination. A significant challenge is the lack of a single perfect method; using a composite reference standard that combines multiple diagnostic methods can be helpful to mitigate this [102]. Intensified efforts to identify early infection biomarkers and differentiate chronic disease from ongoing active infection will be helpful.

Spectrometry

Studies show the different changes in specific metabolites in the plasma of a patient infected with enteric fever and a healthy person. Many gas chromatography-mass spectrometry studies showed clear peaks in 695 metabolites compared to their controls [103], and among them, the top six metabolites (ethanolamine, gluconic acid, monosaccharide, phenylalanine, pipecolic acid, and saccharide) were studied. These showed a clear difference in the plasma of typhoid fever when compared to healthy people and paratyphoid fever

infection. An upregulation of hepcidin causing a fast reduction in serum iron levels and disruption of tryptophan breakdown is also closely related to *S.* Typhi infection [104,105].

Immunological Assay

Identifying different protein markers is also one of the newer approaches to diagnosing enteric fever. An ELISA and immunoblot-based test, the typhoid paratyphoid test (TPTest), compares IgA levels against *S.* Typhi and *S.* Paratyphi membrane components [69,99,106]. In patients with suspected typhoid, other illnesses, and healthy controls, the TPTest is applicable.

Furthermore, immunodominant and immunogenic antigens related to enteric fever have been studied using protein microarrays and immunoaffinity proteomics technology [107-110]. Mass spectrometry-based proteomics usually follows these processes, showing the bacteria bound to the proteins. Other studies have analyzed antigens using liquid chromatography-mass spectrometry with confirmation by western blotting. OmpA, OmpC, PagC, and HlyE are a few of the identified immunogenic antigens of the bacteria.

Molecular Assay-Polymerase Chain Reaction

A newer promising method of diagnosing enteric fever involves identifying and amplifying nucleic acid biomarkers using polymerase chain reaction (PCR), which involves a signature comprised of five host genes, namely STAT1, SLAMF8, PSME2, WARS, and ALDH1A1. This test had a 97% sensitivity and 88% specificity for enteric fever [111]. Microarray hybridization, RNA sequencing, and identifying mRNA using magneto-DNA probes are other various ways of detecting nucleic acid biomarkers.

Various DNA detection using PCR assays majorly focuses on the flagellin gene while using different bases to enhance the sensitivity. The flagellin gene is flic-a for *S.* Paratyphi A and flic-d for *S.* Typhi [112-116]. It appears to be promising not only because of the increased sensitivity and specificity but also because of the ability of PCR to amplify the DNA from dead bacteria.

Early interventions, preventive strategies, and the value of vaccination

Enteric fever requires punctual and efficient resolution to prevent complications, relapse, chronic carrier state, and death. The rising rates of antibiotic resistance worldwide have caused stimulated interest in a comprehensive strategy for the control of the disease. Many efforts are made globally to monitor public health and introduce large-scale immunization programs to mitigate the burden of the disease [117].

Public Health Measures

According to WHO reports in 2017, 2 billion people in Sub-Saharan Africa and Asia still lack access to basic sanitation facilities, and 18% of the worldwide population (1.4 billion people) has no access to handwashing facilities [118]. Typhoid fever control requires a robust surveillance system to estimate the disease load and preventive measures such as water, sanitation, hygiene, and personal hygiene awareness [74]. Providing safe drinking water, adequate sanitation, and safe food handling procedures are critical for preventing enteric fever [76]. To prevent the spread, the importance of handwashing before preparing food, eating, or feeding children is well ascertained [76]. Filtration of drinking water, restricting open defecation, establishing sufficient toilets, and educating people about hygiene behaviors are the other measures that help reduce the disease load [119].

Surveillance Programs

Managing typhoid is sometimes exacerbated by a lack of comprehensive countrywide surveillance in afflicted nations [74]. The Integrated Disease Surveillance Program initiated in 2004 aims to create and enhance a decentralized laboratory-based, computerized disease surveillance system to monitor 22 illnesses (including enteric fever) to identify and respond appropriately [74].

The National Surveillance System for Enteric Fever in India is a recent study in India investigating the prevalence of enteric fever in children aged six months to 15 years. The study uses active case-seeking methods to discover patients who might otherwise go unnoticed by facility-based monitoring systems [120].

The Surveillance of Enteric Fever in Asia Project, initiated by the Sabin Vaccine Institute in the United States, is a hospital-based enteric fever monitoring network in Asian nations, including India, Bangladesh, Indonesia, Pakistan, and Nepal [121]. The retrospective investigation in India yielded information about the disease prevalence, presentation, consequences, and antibiotic resistance trends [119]. This project helps with better preparation of future monitoring systems. Nations should routinely gather data on enteric fever to make evidence-based decisions about managing and preventing enteric fever [121].

The Severe Typhoid Fever Surveillance in Africa program stretched on the infrastructure established as part

of the Typhoid Fever Surveillance in Africa Program study to portray the severity and long-term effects of typhoid fever and invasive nontyphoidal *Salmonella* disease across Africa. Between 2010 and 2014, multiple Sub-Saharan African countries gathered blood culture data and generated typhoid incidence rates from the site [122].

The Strategic Typhoid Alliance Across Africa and Asia consortium conducted a prospective multicomponent epidemiological study in Bangladesh, Nepal, and Malawi in three highly populated urban areas. From June 2016, passive surveillance continued at referral hospitals and primary healthcare facilities. Patients who presented with a fever of two days or longer or a recorded temperature of 38°C were enrolled, and blood cultures were collected. Age-stratified sero surveys were conducted at each research location to estimate the seroincidence of typhoid infection. The resultant seroincidence rates could evaluate and establish upper boundaries for the incidence rates determined from blood culture monitoring. Host responses serve as a proxy for infection incidence, and serosurveys can also detect the probable chronic carriers of *S.* Typhi. To discover stool shedding, people with a robust anti-Vi antibody will do a stool culture [123].

Some of the obstacles that surveillance systems encounter include inaccuracies in reporting fever, errors in diagnostic testing, the prolonged time required to report culture findings, shortage of diagnostic facilities in rural and isolated regions, and insufficiently trained laboratory staff [74].

Identification of various epidemiological features is critical in any surveillance system. Failure to examine these may result in a misunderstanding of disease burden, age distribution, cost of illness, transmission, and antibiotic susceptibility patterns [124].

Typhoid Vaccines

Vaccination is the primary strategy to lessen antibiotic dependency while also protecting against future antimicrobial resistance [125]. The early 1970s chloramphenicol-resistant typhoid outbreak in Mexico prompted a significant focus on vaccine research. It led to the creation of the live oral vaccine, Ty21a, and the non-denatured Vi polysaccharide vaccine [125].

Currently, three kinds of typhoid vaccinations are available for use: live oral Ty21a vaccine, parenteral Vi polysaccharide vaccine, and parenteral typhoid conjugate vaccine (TCV) [126].

Ty21a vaccine: Ty21a is a live vaccination that contains a mutant strain of *S.* Typhi (Ty21a) and is administered orally in three or four doses over five days. There is no approval for use in children under six [127]. The 2018 Cochrane report stated that one year after the Ty21a vaccination, effectiveness was 45%, and 59% after two years [128].

Vi polysaccharide vaccine: The Vi polysaccharide vaccine, developed in the 1980s, is a single-dose injectable vaccination containing purified Vi antigen. There is no approval for children under two, and because the immunological response is generally short-lived, we need to repeat the doses every two to three years [129]. A randomized, double-blind trial involving over 11,000 children in South Africa found 64% effectiveness after 21 months of surveillance [130]. In a study of 131,271 children and adults in China, an efficacy of 69 % was recorded [131]. According to an Indian study, mass vaccination with the Vi vaccine protects unvaccinated community members, with 44% efficacy recorded among non-vaccinated persons residing in vaccination regions. Children aged two to five years old had 80% protection [132]. The advantages of the Vi vaccine include a low side effect profile and convenience of administration (only one injection) [126].

However, following an initial peak post-vaccine, Vi antibody levels gradually declined; one study found that nearly two-thirds of patients had antibody titer below their predicted protective cut-off 27 months after vaccination [133]. Various animal investigations in the 1990s revealed that conjugating the Vi polysaccharide to a protein carrier boosted its immunogenicity [134].

TCVs: The WHO recommends TCVs for use in endemic regions, and they are the preferable vaccination in children as young as six months old [135]. Some examples of TCVs are Vi-rEPA TCV (Vi conjugated to recombinant exoprotein of *Pseudomonas aeruginosa*), Vi-Tetanus Toxoid TCV (Vi conjugated to tetanus toxoid), PedaTyph (Vi conjugated to tetanus toxoid), and Typbar TCV (Vi conjugated to tetanus toxoid) [126].

Vi-rEPA by the National Institutes of Health in the United States went through clinical studies in both the United States and Vietnam. However, they never scaled up the manufacturing to an industrial level [136]. In India, the PedaTyph vaccine and Typbar TCV got the license for use [117].

In a study involving 1,765 Indian children over six months, PedaTyph proved 100% efficient in preventing typhoid. Over 12 months, the vaccination group did not report any typhoid fever cases compared to a typhoid rate of 1.27% in the control group. At 12 months after vaccination, 83% of vaccinated children had significant antibody responses (a fourfold increase from baseline) [137].

Shakya et al. stated in 2019 that Typbar TCV revealed protective vaccination efficacy of 81.6% during a 15-month follow-up period in an interim analysis of a study including over 20,000 Nepali children [138].

There are numerous additional TCVs in various stages of development and those that are previously licensed. Vi-CRM197 from GlaxoSmithKline, a Vi-TT (tetanus toxoid) vaccine from Zydus Cadila, and Vi-DT (diphtheria toxoid) from the International Vaccine Institute are among them [139].

University of Maryland's Center for Vaccine Development generated live, attenuated oral vaccine strains, namely CVD 908, CVD 909, CVD 910, and CVD 915 [140]. The *S.* Typhi Ty2 strain served as the basis for all four strains [140]. CVD 909 was designed to produce Vi polysaccharides and stimulate Vi antibody responses and humoral and cell-mediated immune responses [141]. CVD 909 is an oral primer prior to parenteral Vi polysaccharide vaccine administration. In participants primed with CVD 909, Vi-specific IgA B memory cells were enhanced, suggesting that oral vaccinations might improve immunological memory [141].

While breakthroughs in water and sanitation have abolished endemic typhoid in many areas, numerous countries are still decades away from having enough water and sanitation to avoid typhoid fever. In such cases, antibiotics and vaccines are critical tools in the fight against typhoid fever.

Conclusions

Enteric fever is still a significant cause of morbidity and mortality in developing countries. The diagnostic dilemma in most cases is mainly due to the diverse clinical presentation and lack of accurate diagnostic methods. The organism primarily affects the gastrointestinal tract and reticuloendothelial system. Literature has described multiple mechanisms responsible for the organism's Vi, like *Salmonella* pathogenicity islands, type III secretion mechanism, PAMPs, and secretion of AB toxin.

We have identified various genes responsible for the resistance of drugs like chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. However, the increasing resistance to newer antibiotics is alarming and makes the management of enteric fever more complicated. The choice of antibiotic for enteric fever should be guided by local resistance patterns and susceptibility testing. It's crucial to consult with a healthcare professional or infectious disease specialist who is familiar with the current antibiotic resistance patterns in a specific region. However, combining fluoroquinolone with azithromycin or third-generation cephalosporin with azithromycin has become a recommended approach in some regions. Further research is still needed to comprehend drug resistance and develop new antimicrobials. The early diagnosis of enteric fever helps prompt treatment and prevention of chronic carrier state. New diagnostic methods like Typhidot, Tubex TF, TPTest, spectrometry, and PCR are up-and-coming with increased sensitivity. The benefit of these tests in resource-limited countries is debatable due to their limited accessibility and cost-effectiveness. Preventive strategies include implementing public health measures and immunizing the at-risk population, and proper surveillance plays a vital role in the long-term management of the disease. We recommend further surveillance studies in the endemic areas, which help identify the development of resistant strains and the effectiveness of the management policies.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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