



TYPHOID FEVER IN THE 21ST CENTURY: EPIDEMIOLOGY, ANTIMICROBIAL RESISTANCE, DIAGNOSTICS, VACCINATION STRATEGIES, AND FUTURE THERAPEUTIC APPROACHES

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ABSTRACT

Background: Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S. Typhi*), remains a significant public health challenge in low- and middle-income countries (LMICs), particularly across South Asia, sub-Saharan Africa, and Southeast Asia. Despite advances in sanitation and healthcare infrastructure, the disease continues to exact a heavy toll on vulnerable populations, with an estimated 11–21 million cases occurring annually and approximately 128,000–161,000 deaths reported each year. Children under five years of age bear a disproportionate burden, and the emergence of drug-resistant strains has complicated treatment protocols substantially. **Current Global Burden:** The World Health Organization (WHO) identifies typhoid fever as a priority disease requiring urgent attention in the context of global antimicrobial resistance (AMR). Recent large-scale epidemiological studies, including the Global Enteric Multicenter Study (GEMS) and the Surveillance for Enteric Fever in Asia Project (SEAP), have revised earlier incidence estimates upward, highlighting the inadequacy of passive surveillance systems. Urban slums, peri-urban settings with inadequate sanitation infrastructure, and regions experiencing political instability or humanitarian crises contribute disproportionately to the global disease burden. **Emerging Challenges:** The therapeutic landscape for typhoid fever has been fundamentally altered by the emergence and rapid global dissemination of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *S. Typhi*. The XDR strain, first described from Hyderabad, Pakistan in 2016, carries resistance to first-line antibiotics (chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole), fluoroquinolones, and third-generation cephalosporins, leaving azithromycin and carbapenems as the only reliable oral and injectable treatment options, respectively. This genomic evolution represents one of the most significant public health emergencies in enteric fever management. **Diagnostic Limitations:** Accurate and timely diagnosis of typhoid fever remains constrained by the poor sensitivity of blood culture in field settings, the unreliability of serological tests such as the Widal test in endemic regions, and the lack of affordable point-of-care molecular diagnostics. Emerging technologies including CRISPR-Cas12-based biosensors, loop-mediated isothermal amplification (LAMP), and artificial intelligence-assisted diagnostic platforms offer promising avenues but remain largely unvalidated at scale in resource-limited settings. **Scope of the Review:** This comprehensive review synthesizes evidence from 150 peer-reviewed publications, WHO reports, and epidemiological databases published over the past fifteen years. It examines the current global epidemiology of typhoid fever, elucidates the molecular mechanisms underlying antimicrobial resistance, evaluates the performance characteristics of conventional and emerging diagnostic modalities, assesses the safety and efficacy profiles of currently available vaccines including typhoid conjugate vaccines (TCVs), and explores novel therapeutic strategies including phage therapy, nanomedicine-based drug delivery, and host-directed therapeutic interventions. **Major Findings:** Our review identifies several critical findings. First, the global incidence of typhoid fever remains substantially underestimated due to inadequate surveillance infrastructure. Second, XDR typhoid has evolved through the acquisition of resistance determinants on a single *IncY* plasmid and continues to spread internationally through travel and trade networks. Third, typhoid conjugate vaccines demonstrate 79–82% efficacy in clinical trials and represent a major advancement over unconjugated polysaccharide vaccines, particularly in children under two years of age. Fourth, WASH (Water, Sanitation, and Hygiene) interventions remain the most cost-effective long-term control measure but require sustained political commitment and financing. Fifth, whole-genome sequencing (WGS) of *S. Typhi* isolates has revolutionized our understanding of the pathogen's global phylogeography and resistance evolution. **Research Gaps and Future Perspectives:** Significant knowledge gaps persist regarding the immunological correlates of protection following vaccination, the role of environmental reservoirs in sustaining transmission cycles, and the long-term durability of TCV-induced immunity. Future research must prioritize the development of rapid, affordable, field-deployable diagnostics, next-generation vaccine candidates with broader serological coverage, and novel antimicrobial agents with activity against XDR strains. International collaboration through platforms such as the Global Typhoid Alliance and the Coalition Against Typhoid (CaT) is imperative to accelerate progress across these domains. **Conclusions:** Effective control of typhoid fever in the 21st century demands an integrated, multisectoral approach combining vaccination, improved water and sanitation infrastructure, enhanced antimicrobial stewardship, and strengthened surveillance systems. The global scientific community must intensify efforts to translate genomic and immunological insights into clinically actionable tools that can reach the populations most burdened by this preventable disease.

KEYWORDS: Typhoid fever; *Salmonella Typhi*; antimicrobial resistance; XDR typhoid; typhoid conjugate vaccine; epidemiology; diagnostics; phage therapy; WASH; whole-genome sequencing.

1. INTRODUCTION

1.1 Historical Overview and Discovery

Typhoid fever has been a companion to human civilization since antiquity, with clinical descriptions recognizable in the writings of Hippocrates and accounts of febrile illness among ancient armies. The systematic differentiation of typhoid fever from other "continued fevers" began in earnest during the 19th century, driven by the work of Pierre-Charles-Alexandre Louis, who coined the term "typhoïde" (meaning typhus-like) in 1829 based on pathological observations of intestinal Peyer's patch involvement in fatal cases.^[1] However, the etiological agent remained elusive until 1880, when Karl Joseph Eberth first identified rod-shaped bacilli in the spleen and mesenteric lymph nodes of typhoid patients, providing the initial morphological description of the organism subsequently named *Eberthella typhosa*—later reclassified as *Salmonella enterica* serovar Typhi.^[2]

The bacteriological characterization of *S. Typhi* was advanced considerably by Georg Gaffky, who successfully cultured the organism in 1884, enabling systematic study of its growth characteristics and virulence properties.^[3] The late 19th and early 20th centuries witnessed critical advances in understanding the epidemiology of typhoid fever, most notably through the investigations of John Snow's intellectual successors who traced epidemic outbreaks to contaminated water and food supplies. The role of chronic human carriers exemplified by the infamous case of "Typhoid Mary" (Mary Mallon) in early 20th-century New York was documented and analyzed, establishing the importance of asymptomatic carriage in sustaining endemic transmission cycles.^[4]

The introduction of chloramphenicol in 1948 marked the first effective antibiotic treatment for typhoid fever and transformed clinical management dramatically, reducing case fatality rates from approximately 15–20% to below 1% in treated patients.^[5] However, the emergence of chloramphenicol-resistant *S. Typhi* in Mexico in the early 1970s signaled the beginning of an ongoing evolutionary arms race between the pathogen and the antimicrobial agents employed against it a dynamic that has accelerated dramatically in the contemporary era with the emergence of XDR strains.

1.2 Global Public Health Significance

Typhoid fever occupies a distinctive position among infectious diseases as one of the few major pathogens for which humans constitute the sole reservoir, implying that comprehensive control is theoretically achievable through vaccination combined with improvements in water and sanitation infrastructure. Yet despite this theoretical tractability, typhoid fever persists as a major cause of morbidity and mortality in dozens of countries, reflecting deep structural inequalities in access to clean water, sanitation facilities, and healthcare services.^[6]

The global burden of typhoid fever has been the subject

of considerable reassessment in recent years. Estimates published by the Global Burden of Disease (GBD) Collaborative Network suggest that approximately 14.3 million typhoid cases occurred globally in 2017, with 135,900 deaths—figures that, while representing a substantial decline from earlier decades, still indicate an enormous public health challenge concentrated in LMICs.^[7] More recent probabilistic modelling exercises, incorporating data from active surveillance studies in high-burden settings, suggest that these figures may underestimate true incidence by a factor of two to four, primarily because passive surveillance systems in endemic countries capture only a fraction of total cases, particularly in rural areas and among children who may not access formal healthcare.^[8]

The economic impact of typhoid fever extends beyond direct healthcare costs to include substantial indirect costs through lost productivity, school absenteeism, and long-term sequelae including cognitive impairment in children who experience severe illness or neurological complications. A comprehensive economic analysis conducted across five typhoid-endemic countries estimated the annual cost of typhoid fever at over 7 billion US dollars when all direct and indirect costs were included, with the burden falling disproportionately on households already living in poverty.^[9]

1.3 Rationale for the Review

The past decade has witnessed transformative developments across virtually every dimension of typhoid fever research—from the genomics of antimicrobial resistance to the immunology of vaccine-mediated protection, from the molecular biology of host-pathogen interactions to the implementation science of vaccine rollout programs. These developments have created both new opportunities and new challenges that demand comprehensive synthesis and critical evaluation.^[10]

The global emergence and spread of XDR *S. Typhi*, first documented in Pakistan's Sindh province in 2016 and subsequently detected on multiple continents through travel-related cases, has fundamentally altered the clinical and public health calculus of typhoid fever management.^[11] The genomic characterization of this strain, carried out through international collaborative networks including the Global Typhoid Genomics Consortium, has revealed a sophisticated resistance architecture that combines resistance mechanisms acquired across evolutionary time with recently acquired plasmid-borne resistance to extended-spectrum beta-lactamases.^[12] Understanding this architecture is essential for designing rational treatment protocols and surveillance systems.

Simultaneously, the approval and implementation of typhoid conjugate vaccines—particularly Typhbar-TCV and PedaTyph by WHO-prequalified manufacturers and the subsequent rollout programs in Pakistan, Zimbabwe,

and other endemic countries represent a major advance in prevention that requires rigorous evaluation of real-world effectiveness, immunological durability, and implementation challenges.^[13] This review integrates evidence across these domains to provide a current, comprehensive, and critically analyzed synthesis of the typhoid fever field.

1.4 Objectives of the Review

This review pursues four primary objectives: (1) to summarize recent advances in the epidemiology, molecular biology, and clinical management of typhoid fever with particular emphasis on developments since 2015; (2) to analyze the mechanisms, evolution, and public health implications of antimicrobial resistance in *S. Typhi* with detailed attention to XDR strains; (3) to critically evaluate the diagnostic and vaccine technologies currently available and under development; and (4) to identify priority research gaps and propose a framework for future investigation that can accelerate progress toward typhoid elimination.^[14]

2. METHODOLOGY OF LITERATURE REVIEW

2.1 Search Strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive, multi-database literature search was conducted during the period January 2023 through March 2025, encompassing publications from 2010 to the present. The primary electronic databases searched included PubMed/MEDLINE, Scopus, Web of Science, Embase, and Google Scholar. Additional targeted searches were performed on the WHO Institutional Repository for Information Sharing (IRIS), the Cochrane Library, and ClinicalTrials.gov for unpublished or ongoing studies.^[15]

Search terms were developed using a combination of Medical Subject Headings (MeSH) terms and free-text keywords organized around four primary thematic domains: (1) the pathogen and disease "*Salmonella Typhi*," "typhoid fever," "enteric fever," "paratyphoid"; (2) antimicrobial resistance "multidrug resistance," "XDR typhoid," "antimicrobial resistance," "fluoroquinolone resistance," "extended-spectrum beta-lactamase"; (3) diagnostics "typhoid diagnosis," "blood culture," "Widal test," "PCR typhoid," "point-of-care diagnostics"; and (4) prevention and treatment "typhoid vaccine," "typhoid conjugate vaccine," "Vi polysaccharide vaccine," "WASH," "antibiotic stewardship".^[16] Boolean operators (AND, OR, NOT) were employed to combine search terms systematically, and citation tracking of key articles was performed to identify additional relevant studies not captured by database searches.

2.2 Inclusion and Exclusion Criteria

Articles were included if they: (1) were published in peer-reviewed journals between January 2010 and March 2025; (2) reported original research data, systematic

reviews, meta-analyses, or authoritative narrative reviews relevant to typhoid fever epidemiology, pathogenesis, diagnosis, treatment, or prevention; (3) were published in the English language; and (4) provided sufficient methodological detail to allow critical appraisal of findings. Studies from all geographic settings were included to capture the global scope of typhoid fever research, although particular attention was paid to studies from endemic regions in South Asia, Southeast Asia, and sub-Saharan Africa.^[17]

Articles were excluded if they: (1) were published solely as conference abstracts without accompanying peer-reviewed full text; (2) focused exclusively on *Salmonella Paratyphi* or non-typhoidal *Salmonella* without relevance to *S. Typhi*; (3) were duplicate publications; (4) reported case series of fewer than five patients without generalizable findings; or (5) were assessed as having critical methodological flaws that rendered their conclusions unreliable. A total of 847 records were initially identified through database searches; following removal of duplicates ($n=189$) and screening of titles and abstracts ($n=461$ excluded), 197 full-text articles were assessed for eligibility, of which 150 were included in this review.^[18]

2.3 Quality Assessment

The methodological quality of included studies was assessed using standardized tools appropriate to each study design. Observational studies were evaluated using the Newcastle-Ottawa Scale, randomized controlled trials using the Cochrane Risk of Bias tool, and diagnostic accuracy studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) framework. Systematic reviews and meta-analyses were appraised using the AMSTAR-2 checklist. Studies rated as having critical risk of bias across all domains were excluded; those with some concerns regarding specific bias domains were included but their limitations noted and weighted accordingly in the synthesis.^[19]

3. ETIOLOGY AND PATHOGENESIS

3.1 Taxonomy and Microbiology of *Salmonella Typhi*

Salmonella enterica serovar *Typhi* (*S. Typhi*) is a Gram-negative, facultatively anaerobic, non-spore-forming, motile bacillus belonging to the family Enterobacteriaceae. The organism measures approximately 1–3.5 μm in length and 0.5–0.8 μm in width, possesses peritrichous flagella that confer motility, and expresses a polysaccharide capsule the Vi antigen that serves as a critical virulence determinant and immunological target for subunit vaccines.^[20] Unlike most other *Salmonella* serovars, *S. Typhi* is host-restricted to humans, a property attributable to its possession of specific virulence factors adapted for survival within the human immune microenvironment and its lack of key adhesins necessary for efficient colonization of the intestinal mucosa of non-human hosts.

The genome of *S. Typhi* consists of a single circular

chromosome of approximately 4.8 Mb, encoding approximately 4,600 predicted genes, with a GC content of approximately 52%. Comparative genomic analyses have revealed that the *S. Typhi* genome contains numerous pseudogenes approximately 200 compared to fewer than 40 in the closely related non-typhoidal *Salmonella* serovars a pattern consistent with ongoing genome degradation associated with host restriction and the reduced selective pressure on genes not required in the specialized human host niche.^[21] This phenomenon of reductive evolution is paralleled by observations in other obligate human pathogens such as *Yersinia pestis* and *Mycobacterium leprae*, and suggests that *S. Typhi* has undergone relatively recent adaptation to human-specific pathogenesis.

3.2 Mechanism of Infection and Intracellular Survival

The infectious process of typhoid fever is initiated by the ingestion of food or water contaminated with *S. Typhi*, typically at doses of 10^5 to 10^9 bacteria in immunocompetent adults, although experimental human challenge studies have demonstrated that doses as low as 10^3 organisms can establish infection under favorable conditions.^[22] Following ingestion, *S. Typhi* must survive the hostile environment of the stomach, where acidic pH (typically 1.5–3.5) kills the majority of ingested bacteria. Survival is facilitated by the acid tolerance response (ATR), a transcriptionally regulated adaptive program that upregulates multiple acid resistance mechanisms including the arginine- and glutamate-dependent acid resistance systems.

Upon reaching the small intestine, *S. Typhi* encounters the specialized epithelial cells overlying lymphoid follicles known as microfold (M) cells, which serve as the primary portal of entry into the host tissue. M cells sample luminal antigens and microorganisms through transcytosis, delivering them to the subepithelial space where dendritic cells and macrophages of the lamina propria initiate immune responses. *S. Typhi* exploits this normal immunosurveillance function to gain access to subepithelial tissues, employing a type III secretion system (T3SS) encoded on *Salmonella* Pathogenicity Island 1 (SPI-1) to inject effector proteins into host cell cytoplasm and trigger bacterial engulfment.^[23]

Following uptake into macrophages and dendritic cells, *S. Typhi* resides within a specialized vacuolar compartment known as the *Salmonella*-containing vacuole (SCV), which serves as an intracellular replicative niche. The bacterium actively manipulates SCV biogenesis through the actions of a second type III secretion system encoded on SPI-2, which is induced under the low-pH, low-phosphate, low-magnesium conditions of the mature SCV.^[24] SPI-2 effectors collectively serve to prevent fusion of the SCV with lysosomes, redirect vesicular trafficking to provide nutrients to the bacterium, and modulate innate immune signaling pathways to prevent bactericidal responses. This intracellular survival strategy

enables *S. Typhi* to disseminate through the reticuloendothelial system via infected macrophages, reaching the liver, spleen, bone marrow, and mesenteric lymph nodes—a process that correlates clinically with the onset of systemic symptoms during the second week of infection.

3.3 Host–Pathogen Interaction and Immune Evasion

The ability of *S. Typhi* to establish systemic infection in the face of potent host immune defenses reflects a sophisticated array of immune evasion strategies. The Vi polysaccharide capsule—a key virulence factor unique to *S. Typhi* among *Salmonella* serovars—serves multiple immune evasion functions. It masks underlying surface antigens including lipopolysaccharide (LPS) O-antigen from pattern recognition receptors, thereby reducing stimulation of toll-like receptor 4 (TLR4)-mediated innate immune signaling; it inhibits complement-mediated opsonization by interfering with C3b deposition; and it provides physical protection against antimicrobial peptides and oxidative bursts.^[25]

S. Typhi has also evolved mechanisms to subvert adaptive immune responses. The bacterium produces a cytolethal distending toxin (typhoid toxin, encoded by *cdtB* and *pltA* genes) that induces DNA damage in host cells, inhibiting T cell proliferation and potentially contributing to the immunosuppression characteristic of severe typhoid fever.^[26] Additionally, *S. Typhi* modulates dendritic cell maturation and cytokine production to suppress Th1-polarized responses which are critical for macrophage activation and bacterial clearance while promoting anti-inflammatory cytokine profiles. This immunological manipulation creates a window of reduced immune effector function during the bacteremic phase of infection that enables widespread bacterial dissemination before effective adaptive responses can be mounted.

3.4 Molecular Basis of Virulence

The virulence of *S. Typhi* is encoded by a modular genetic architecture comprising multiple pathogenicity islands, mobile genetic elements, and chromosomally encoded virulence genes. The five *Salmonella* pathogenicity islands (SPI-1 through SPI-5) collectively encode over 30 virulence effector proteins that orchestrate bacterial invasion, intracellular survival, and immune evasion.^[27] SPI-1 encodes the invasion-associated T3SS and its effectors (*SipA*, *SipB*, *SopB*, *SopE/E2*), while SPI-2 encodes the intracellular-phase T3SS with effectors critical for SCV maintenance (*SseF*, *SseG*, *SifA*). SPI-3 through SPI-5 encode additional colonization factors, magnesium transporters, and effectors that fine-tune host cell interactions.

The *viaB* locus, which encodes both the Vi antigen biosynthetic machinery and its transcriptional regulator *TviA*, plays a central role in coordinating *S. Typhi* virulence gene expression. *TviA* acts as a global virulence regulator that represses SPI-1-associated

invasion genes while upregulating Vi antigen expression, effectively switching the bacterium from an invasive phenotype appropriate for initial intestinal invasion to a stealthy, non-inflammatory phenotype suited for systemic dissemination.^[28] This transcriptional toggle represents an elegant adaptive strategy that minimizes inflammatory signaling during the bacteremic phase, when bacterial burden is highest and immune detection would be most threatening to bacterial survival.

4. EPIDEMIOLOGY AND GLOBAL DISEASE BURDEN

4.1 Geographic Distribution and Incidence

The global distribution of typhoid fever is highly heterogeneous, with the overwhelming majority of cases concentrated in LMICs across South Asia, Southeast Asia, sub-Saharan Africa, and Latin America. South Asia bears the greatest burden, accounting for approximately 70% of global cases; within this region, India, Pakistan, and Bangladesh are the most affected countries.^[29] The Surveillance for Enteric Fever in Asia Project (SEAP), which conducted prospective population-based surveillance across five Asian countries from 2016 to 2019, estimated crude incidence rates ranging from 180-494 cases per 100,000 person-years in different study sites, with the highest rates observed in urban low-income neighborhoods in Dhaka, Bangladesh, and Kolkata, India.^[30]

In sub-Saharan Africa, the epidemiological picture of typhoid fever has historically been obscured by the absence of adequate laboratory infrastructure for blood culture confirmation and the overlapping clinical presentation of typhoid with malaria, which remains hyperendemic across much of the continent. Active surveillance programs conducted under the auspices of the Severe Typhoid in Africa Program (SETA) have revealed substantial incidence rates particularly among children aged 2-14 years in countries including Malawi, Kenya, Ghana, and Nigeria, with estimates suggesting that sub-Saharan Africa may account for 25–30% of global typhoid cases.^[31] The Democratic Republic of Congo, Ethiopia, and South Sudan have been identified as particularly high-burden settings based on healthcare facility surveillance data.

4.2 Risk Factors and Social Determinants

The risk factors for typhoid fever are intimately connected to deficits in water, sanitation, and hygiene (WASH) infrastructure a reality that concentrates disease burden among the world's poorest and most marginalized populations. Access to safely managed drinking water, defined by WHO/UNICEF as water from an improved source that is accessible on-premises, available when needed, and free from fecal and priority chemical contamination, remains unavailable for approximately 785 million people globally.^[32] In typhoid-endemic settings, consumption of water from unimproved or inadequately treated sources, street food and raw vegetables irrigated with wastewater, and raw shellfish

from fecally contaminated water bodies have been consistently identified as major risk factors for *S. Typhi* infection in case-control studies.

Household and community-level factors associated with increased typhoid risk include absence of soap and water for handwashing at critical moments (after defecation and before food preparation), open defecation or use of shared unimproved latrines, flooding of household environs (particularly in densely populated urban settings during monsoon seasons), and household crowding that facilitates person-to-person transmission via fecal-oral routes.^[33] Individual-level risk factors include age (children 2–14 years are at highest risk in highly endemic settings due to incomplete immunity from prior exposure), HIV infection and other immunocompromising conditions, achlorhydria or use of proton pump inhibitors, and travel to endemic regions without prior vaccination.

4.3 Typhoid in Special Populations

Children under five years of age represent a particularly vulnerable population in typhoid-endemic settings. The clinical presentation of typhoid in young children frequently differs from the classic adult picture described in textbooks: fever may be less reliably present, gastrointestinal symptoms may predominate, and complications including febrile seizures occur with greater frequency.^[34] Moreover, young children are more likely to develop severe disease and to suffer long-term sequelae including cognitive impairment related to neurological complications. The relative protective effect of Vi-antigen-directed immunity in older children and adults—acquired through natural exposure does not extend to very young children who have not yet had sufficient environmental exposure to mount protective immune responses.

Typhoid fever during pregnancy carries substantial risks for both mother and fetus. Maternal infection is associated with a three-fold increased risk of spontaneous abortion and a two-fold increased risk of preterm delivery compared to uninfected pregnant women in endemic settings.^[35] The pathophysiological mechanisms underlying these adverse obstetric outcomes include fever-induced uterine contractions, transplacental bacteremia with potential for fetal infection, and cytokine-mediated effects on trophoblast function. Treatment of typhoid in pregnancy is complicated by the contraindication of fluoroquinolones and chloramphenicol in the first trimester and the relative safety concerns regarding azithromycin, leaving intravenous ceftriaxone or ampicillin (for susceptible strains) as the treatments of choice.

4.4 Impact of Climate Change and Urbanization

The relationship between climate variability, urbanization patterns, and typhoid fever transmission is gaining increasing recognition as a critical dimension of the disease's future epidemiology. Mathematical

modeling studies have demonstrated significant associations between rainfall patterns—particularly intense precipitation events and subsequent flooding—and typhoid fever incidence in South Asian cities, likely reflecting contamination of drinking water sources and disruption of sanitation infrastructure.^[36] In the context of climate change, increases in the frequency and intensity of extreme weather events including floods and cyclones are projected to amplify typhoid transmission in already-vulnerable populations, particularly in low-lying coastal cities and flood-prone river valleys of South and Southeast Asia.

Rapid unplanned urbanization represents a parallel structural driver of typhoid fever burden in LMICs. The growth of informal urban settlements (slums), where population densities may exceed 100,000 persons per square kilometer, creates conditions of extreme WASH deprivation that sustain intense typhoid transmission cycles.^[37] The United Nations projects that the global urban population will increase by approximately 2.5 billion people by 2050, with the majority of this growth concentrated in urban agglomerations in typhoid-endemic regions of Africa and Asia. Without commensurate investment in urban WASH infrastructure, this demographic trajectory portends a sustained or worsening typhoid burden in coming decades.

5. CLINICAL MANIFESTATIONS AND COMPLICATIONS

5.1 Classic Clinical Presentation

The classic clinical presentation of typhoid fever unfolds over three to four weeks in a characteristic pattern that, while subject to modification by age, immune status, infecting dose, and antibiotic treatment, remains recognizable in its broad outlines. Following an incubation period of typically 7–14 days (range 3–60 days), the illness begins with a gradual onset of fever that rises in a stepwise fashion over the first week to reach sustained temperatures of 39–40°C or higher.^[38] This first week is characterized by nonspecific constitutional symptoms including malaise, anorexia, headache, and generalized myalgia that frequently leads to misdiagnosis as viral illness or malaria in endemic settings.

The second week of illness is characterized by the development of more specific clinical features. Relative bradycardia a dissociation between fever height and heart rate—is observed in up to 30% of cases and provides a useful clinical clue to diagnosis. Rose spots, transient salmon-colored maculopapular lesions typically 2–4 mm in diameter, appear in crops on the trunk in approximately 10–30% of patients with lightly pigmented skin but are rarely identified in dark-skinned individuals.^[39] Hepatosplenomegaly develops in approximately 30–50% of patients, reflecting reticuloendothelial involvement. Abdominal distension, constipation (more common in adults) or diarrhea (more common in children), and a coated tongue are frequently

observed.

5.2 Severe Complications

Intestinal perforation represents the most feared complication of typhoid fever, occurring in 0.8–3% of hospitalized patients in most series but reaching rates of 10–15% in some African settings where delayed diagnosis and treatment are common.^[40] Perforation results from transmural necrosis of the ileum at sites of Peyer's patch inflammation, typically during the third week of illness when immune-mediated tissue destruction is at its peak. It presents with sudden onset of severe abdominal pain, rigidity, and signs of peritonitis, and carries a case fatality rate of 10–32% even with surgical intervention and intensive supportive care. Risk factors for perforation include delayed diagnosis, MDR or XDR strain infection, malnutrition, and concomitant helminthic infestation.

Neuropsychiatric manifestations of typhoid fever collectively termed "typhoid encephalopathy"—occur in 10–40% of hospitalized patients in some series and encompass a spectrum from mild confusion and delirium to frank psychosis, cerebellar ataxia, seizures, and coma.^[41] The pathophysiology of typhoid encephalopathy remains incompletely understood but likely involves direct neurotropism of *S. Typhi*, cytokine-mediated disruption of the blood-brain barrier, endotoxin-related cerebral edema, and metabolic encephalopathy secondary to hepatic dysfunction. Dexamethasone has been shown in a landmark randomized controlled trial conducted in Jakarta to reduce mortality in patients with severe typhoid fever presenting with altered consciousness or shock, although subsequent studies have yielded conflicting results regarding the generalizability of this benefit.^[42]

5.3 Chronic Carriage and Long-Term Consequences

Approximately 1–6% of patients with acute typhoid fever develop chronic gallbladder carriage, defined as continued excretion of *S. Typhi* in feces for more than 12 months following acute infection.^[43] Chronic carriers harbor bacteria in the bile duct or gallbladder mucosa, frequently in association with gallstones that provide protected biofilm niches resistant to antibiotic penetration. Chronic carriers do not experience ongoing symptoms but serve as a critical reservoir sustaining the environmental cycle of *S. Typhi* in endemic communities. Risk factors for chronic carriage include female sex, older age at infection, and the presence of cholelithiasis. The association between chronic *S. Typhi* carriage and gallbladder carcinoma mediated through bacterial induction of pro-inflammatory and genotoxic mechanisms has been strengthened by several recent molecular epidemiological studies.

6. DIAGNOSTIC APPROACHES

6.1 Conventional Microbiological Diagnostics

Blood culture remains the reference standard for laboratory diagnosis of typhoid fever despite its well-recognized limitations of sensitivity, accessibility, and

turnaround time. The sensitivity of blood culture in typhoid fever is highly variable, ranging from 40–80% in most clinical series, and depends on multiple factors including the volume of blood cultured (sensitivity increases substantially from approximately 50% with 2 mL to over 70% with 10–15 mL), the timing of sampling relative to disease onset (sensitivity is highest during the first week of illness before significant antibiotic use), prior antibiotic administration (which reduces sensitivity dramatically), and the quality of culture media and incubation conditions available.^[44] In resource-limited settings where automated continuously-monitored blood culture systems are unavailable, manual culture methods with 5–10 day incubation periods further delay diagnosis and may suppress positivity in cultures initiated after antibiotic therapy.

Bone marrow culture achieves the highest sensitivity (80–95%) of any diagnostic modality for typhoid fever and retains this sensitivity even after several days of antibiotic treatment, reflecting the high bacterial density within bone marrow macrophages and the slower penetration of antibiotics into this compartment.^[45] However, the invasive nature of bone marrow aspiration, the specialized training required, and patient acceptability concerns limit its use to research settings and referral hospitals rather than routine clinical practice. Stool and urine cultures have lower sensitivity than blood culture during the first two weeks of illness but may be positive when blood cultures have become negative following antibiotic treatment, and therefore have utility in late-presenting cases.

6.2 Serological Diagnostics

The Widal agglutination test, developed by Georges-Fernand Widal in 1896, measures agglutinating antibodies against *S. Typhi* somatic (O) and flagellar (H) antigens and has been used for over a century as a diagnostic tool in resource-limited settings where blood culture is unavailable.^[46] Despite its widespread use, the Widal test has been subject to persistent criticism based on its poor and highly variable sensitivity (38–97%) and specificity (25–100%) across different endemic settings. These performance limitations reflect multiple confounders: background seropositivity in endemic populations from prior infection or vaccination obscures interpretation of single-titer measurements; cross-reactive antibodies from non-typhoidal *Salmonella* infections, malaria, dengue, and other febrile illnesses generate false-positive results; and the antibody response to acute

S. Typhi infection is too slow to be diagnostically useful in early illness. WHO guidance explicitly discourages sole reliance on the Widal test for diagnosis.

More recently developed point-of-care rapid diagnostic tests (RDTs) including the Typhidot test (detection of IgM and IgG antibodies against a 50 kDa outer membrane protein of *S. Typhi*) and the TUBEX TF test (competitive

inhibition immunoassay detecting anti-O9 antibodies against LPS) have been evaluated extensively in endemic settings.^[47] Meta-analyses of published diagnostic accuracy studies report pooled sensitivities of 69–84% and specificities of 79–89% for these RDTs when evaluated against blood culture as reference standard. While these performance characteristics represent an improvement over the Widal test, they remain insufficient for confident clinical decision-making in individual patients, and their performance may be further degraded in settings with high background seroprevalence.

6.3 Molecular Diagnostics

PCR-based diagnostic methods have been evaluated as alternatives to culture and serology that could offer superior sensitivity, specificity, and speed. Conventional PCR assays targeting conserved *S. Typhi*-specific genomic sequences—including the Vi antigen biosynthesis gene *viaB*, the H1-d flagellin gene *fliC*, and ribosomal sequences—have demonstrated sensitivities of 67–95% and specificities of 89–100% in published studies, though with significant variation attributable to differences in sample type, bacterial load at time of sampling, and DNA extraction methods.^[48] Real-time PCR platforms have improved upon conventional PCR in terms of speed, sensitivity, and capacity for quantitative assessment of bacteremia the latter being potentially informative regarding disease severity.

Whole-blood PCR, which bypasses the selective culture step and directly amplifies *S. Typhi* DNA from lysed blood samples, has emerged as a particularly promising approach for diagnosis under conditions where blood culture sensitivity is compromised by prior antibiotic exposure. Comparative studies from Bangladesh and Nepal have demonstrated sensitivities of 79–94% for whole-blood PCR versus 47–73% for concurrent blood culture in the same patients, suggesting that direct molecular detection can capture a substantially larger fraction of true positive cases.^[49] However, PCR-based methods require dedicated laboratory infrastructure, trained personnel, and quality-controlled reagent supply chains that remain unavailable in most primary healthcare settings in LMICs, limiting their current impact on routine case management.

6.4 Emerging Diagnostic Technologies

The past five years have witnessed rapid development of novel diagnostic technologies that hold promise for transforming typhoid fever diagnosis in resource-limited settings. CRISPR-Cas12a-based diagnostic platforms, which combine the isothermal amplification of target DNA with highly specific detection mediated by guide RNA-directed Cas12 endonuclease activity, have been adapted for *S. Typhi* detection with reported sensitivities and specificities approaching 100% in laboratory evaluations.^[50] These platforms can be configured as lateral-flow assays readable with the naked eye, potentially enabling point-of-care deployment without

laboratory infrastructure. However, peer-reviewed clinical validation studies using well-characterized clinical specimens from typhoid-endemic settings remain sparse, and the performance of CRISPR-based assays in the presence of complex biological matrices (whole blood, stool) with varied inhibitor profiles requires further characterization.

Biosensor-based detection approaches leveraging aptamer technology, electrochemical impedance spectroscopy, and surface-enhanced Raman spectroscopy have been described for *S. Typhi* detection in proof-of-concept studies, with some platforms demonstrating

detection limits in the femtomolar range using cultured bacteria.^[51] Artificial intelligence-assisted image analysis has been applied to interpret microscopy images and rapid test results with reduced inter-reader variability, and machine learning algorithms trained on clinical and demographic data have been explored as decision-support tools for typhoid diagnosis in settings with high background febrile illness prevalence. While these technologies represent exciting proof-of-concept advances, substantial translational work remains before they can contribute meaningfully to routine clinical practice in endemic settings.

Table 1: Comparative Evaluation of Typhoid Fever Diagnostic Methods.

Diagnostic Method	Sensitivity (%)	Specificity (%)	Time to Result	Cost	Feasibility (LMIC)
Blood Culture	40–80	95–99	3–7 days	Low–Moderate	Limited
Bone Marrow Culture	80–95	96–100	3–7 days	High	Reference only
Widal Test	38–97	25–100	2–4 hours	Very Low	Widely available
Typhidot RDT	69–84	79–89	1–2 hours	Low	Good
TUBEX TF	69–78	80–89	2 hours	Low–Moderate	Good
Conventional PCR	67–95	89–100	4–6 hours	Moderate	Tertiary only
Real-Time PCR	79–94	91–100	3–4 hours	High	Limited
CRISPR-based Assay	~95–100*	~98–100*	1–2 hours	Moderate	Under evaluation

*Laboratory evaluation only; clinical field validation pending. LMIC = Low- and Middle-Income Country; RDT = Rapid Diagnostic Test.

7. ANTIMICROBIAL RESISTANCE

7.1 Historical Evolution of Resistance

The history of antimicrobial resistance in *S. Typhi* is a chronicle of sequential therapeutic failures driven by the remarkable genomic plasticity of the organism and the intense selective pressure exerted by widespread antibiotic use in endemic settings. Chloramphenicol, introduced as the first effective typhoid therapy in 1948, maintained its utility for over two decades before the emergence of chloramphenicol-resistant *S. Typhi* strains in Mexico in 1972, where they caused a devastating epidemic affecting approximately 100,000 persons with a case fatality rate exceeding 15%.^[52] Resistance was mediated by a self-transferable IncHI1 plasmid carrying the *cat* gene encoding chloramphenicol acetyltransferase, which rapidly disseminated to *S. Typhi* populations in Asia, the Middle East, and Africa over the subsequent decade.

The emergence of multidrug-resistant (MDR) *S. Typhi*—defined as resistance to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, the three first-line agents recommended for typhoid treatment before fluoroquinolone availability was documented in the late 1980s and early 1990s, initially from the Indian subcontinent and subsequently spreading globally.^[53] MDR strains carried a complex resistance determinant initially borne on an IncHI1 plasmid that subsequently integrated into the chromosome in many lineages. By the mid-1990s, MDR strains accounted for the majority of *S.*

Typhi isolates from several endemic countries, effectively rendering first-line therapies obsolete and shifting clinical practice toward fluoroquinolones as the treatment of choice.

Fluoroquinolone resistance emerged through a distinct mechanism—chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (*gyrA*, *gyrB*) and topoisomerase IV (*parC*, *parE*) genes rather than through plasmid-mediated mechanisms, reflecting the low frequency of horizontal transfer of chromosomal mutations versus plasmid-borne resistance genes. Single mutations in *gyrA*, particularly the substitutions Asp87→Asn and Ser83→Phe/Tyr, confer high-level nalidixic acid resistance and reduced susceptibility to fluoroquinolones with minimum inhibitory concentrations in the susceptible-intermediate range.^[54] Accumulation of multiple QRDR mutations leads to high-level fluoroquinolone resistance with clinical treatment failure, a scenario increasingly documented from Pakistan, India, and Bangladesh from the late 2000s onward.

7.2 Extensively Drug-Resistant XDR Typhoid

The emergence of extensively drug-resistant (XDR) typhoid, defined as resistance to first-line antibiotics (chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole), fluoroquinolones, and extended-spectrum cephalosporins (primarily ceftriaxone), represents the most serious development in typhoid fever

therapeutics in decades. The XDR strain was first systematically described in 2018 by Klemm and colleagues following an epidemic beginning in Hyderabad, Sindh Province, Pakistan in November 2016 that subsequently spread to involve all four provinces of Pakistan and Azad Kashmir.^[55]

Whole-genome sequencing analyses of XDR isolates demonstrated that resistance to ceftriaxone (and other extended-spectrum cephalosporins) was conferred by an blaCTX-M-15 gene encoding a Class A extended-spectrum beta-lactamase (ESBL), carried on a novel IncY plasmid designated pHCM2 in the original characterization. This same plasmid also carried genes conferring resistance to fluoroquinolones through a plasmid-mediated quinolone resistance (PMQR) mechanism (qnrS gene), aminoglycosides, tetracyclines, and sulfonamides.^[56] The acquisition of this resistance plasmid by an already-MDR H58 (now designated 4.3.1) *S. Typhi* strain background effectively created a strain with resistance to all oral antibiotics previously recommended for typhoid treatment except azithromycin, leaving clinicians dependent on carbapenems for severe XDR typhoid cases requiring parenteral therapy.

The global dissemination of XDR *S. Typhi* from Pakistan has been documented through genomic epidemiology of travel-associated cases in the United Kingdom, United States, Canada, Australia, Denmark, and multiple other countries.^[57] The detection of XDR typhoid in travelers who visited Pakistan has prompted updated travel medicine recommendations in several countries, including the specific recommendation for typhoid conjugate vaccination prior to travel to Pakistan. The persistence and geographic expansion of XDR typhoid within Pakistan, where it now accounts for over 70% of blood-culture-confirmed typhoid cases in some surveillance sites, reflects the challenge of interrupting transmission in high-burden settings through treatment alone without complementary vaccination and WASH interventions.

7.3 Mechanisms of Resistance

The molecular mechanisms underlying antimicrobial resistance in *S. Typhi* encompass multiple distinct biochemical pathways that collectively confer resistance to structurally diverse antibiotic classes. Beta-lactam resistance is mediated primarily by beta-lactamase enzymes, with the plasmid-borne TEM-1 (narrow spectrum) and CTX-M-15 (extended spectrum) enzymes being the most clinically significant in *S. Typhi*.^[58] The CTX-M family of ESBLs is of particular concern because of their broad substrate spectrum encompassing third- and fourth-generation cephalosporins, their propensity for dissemination on conjugative plasmids, and the presence of promoter mutations and gene amplification events that drive high-level expression. AmpC-type cephalosporinases, which confer resistance

to cephalosporins including ceftriaxone and are not inhibited by standard beta-lactamase inhibitors such as clavulanic acid, have also been reported in *S. Typhi* isolates from Pakistan and Vietnam.

Fluoroquinolone resistance in *S. Typhi* involves multiple cooperative mechanisms. Target site mutations in *gyrA* (most commonly Ser83Phe and Asp87Asn) reduce binding affinity of fluoroquinolones to the DNA gyrase complex. Plasmid-mediated quinolone resistance (PMQR) genes including *qnrS*, *qnrB*, and *aac(6)-Ib-cr* protect the enzyme target and modify the drug itself through acetylation.^[59] Efflux pump overexpression, particularly of the AcrAB-TolC pump system regulated by the MarA, SoxS, and RamA transcriptional activators, reduces intracellular fluoroquinolone concentrations below inhibitory thresholds. The simultaneous presence of multiple fluoroquinolone resistance mechanisms in XDR strains elevates minimum inhibitory concentrations to levels that preclude effective clinical use of this drug class.

7.4 Resistance Surveillance and Genomic Epidemiology

The establishment of robust international surveillance systems for tracking *S. Typhi* antimicrobial resistance has been recognized as a public health priority, and significant infrastructure has been developed in recent years to address this need. The Global Typhoid Genomics Consortium, established in 2018, has collated whole-genome sequence data from over 13,000 *S. Typhi* isolates collected from 70 countries spanning more than a century of isolate collection, creating an unprecedented resource for phylogeographic analysis and resistance surveillance.^[60] This genomic database has enabled detailed reconstruction of the evolutionary pathways through which resistance has been acquired and has documented multiple independent acquisitions of similar resistance determinants by different *S. Typhi* lineages, reflecting the high probability of horizontal gene transfer in environments with intense antibiotic selection pressure.

Table 2: Evolution of Antimicrobial Resistance in Salmonella Typhi.

Era	Resistance Pattern	Mechanism	Geographic Origin	Clinical Impact
1972–1989	Chloramphenicol-R	cat gene (plasmid IncHI1)	Mexico, then Asia	Loss of first-line drug
1989–2000	MDR (Chlor/Amp/TMP-SMX)	IncHI1 plasmid / chromosomal integration	South Asia	Shift to fluoroquinolones
2000–2015	Fluoroquinolone Reduced Susceptibility	gyrA/parC QRDR mutations	South Asia, worldwide	Increased treatment failure
2015–Present	XDR (MDR + FQ + 3GC)	IncY plasmid (blaCTX-M-15, qnrS)	Pakistan (2016)	Carbapenem-dependent therapy
Emerging	Azithromycin resistance	acrB mutations (R717Q/L)	Bangladesh, India	Loss of oral XDR treatment

Abbreviations: R = Resistant; Amp = Ampicillin; TMP-SMX = Trimethoprim-Sulfamethoxazole; FQ = Fluoroquinolones; 3GC = Third-generation Cephalosporins; QRDR = Quinolone Resistance-Determining Region.

8. TREATMENT AND THERAPEUTIC ADVANCES

8.1 Current Antibiotic Treatment Guidelines

The selection of antibiotic therapy for typhoid fever is now guided primarily by the antimicrobial susceptibility profile of locally circulating strains, which must be established through active surveillance rather than assumed based on historical patterns.^[61] For drug-susceptible typhoid fever, the fluoroquinolone ciprofloxacin or ofloxacin given for 5-7 days remains the preferred treatment in most guidelines, achieving clinical cure in over 95% of cases with susceptible strains. Azithromycin given for 5–7 days represents an effective oral alternative, particularly in pediatric patients, and has demonstrated non-inferiority to fluoroquinolones in randomized trials conducted in Bangladesh and Nepal for uncomplicated typhoid fever caused by nalidixic acid-susceptible isolates.

For MDR typhoid fever (resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole), fluoroquinolones remain effective when isolates lack QRDR mutations, and azithromycin or ceftriaxone are appropriate alternatives. For XDR typhoid fever, the treatment algorithm is substantially more constrained.^[62] Azithromycin at doses of 20 mg/kg/day (maximum 1 g/day) for 7 days represents the sole recommended oral option, whereas intravenous ertapenem or meropenem are required for severe disease or patients unable to tolerate oral medication. The use of carbapenems for community-acquired typhoid fever infections largely occurring in pediatric populations in LMICs—raises profound concerns regarding antimicrobial stewardship, healthcare costs, and the accelerated selection of carbapenem-resistant organisms.

Reports of azithromycin-resistant *S. Typhi* from Bangladesh, India, and Nepal since 2019 have introduced the alarming possibility of pan-drug-resistant typhoid fever, for which no reliably effective oral antibiotic treatment exists.^[63] Azithromycin resistance in *S. Typhi* has been associated with mutations in the *acrB* gene of the AcrAB-TolC efflux pump system, specifically the R717Q and R717L substitutions that increase the drug

efflux capacity and elevate minimum inhibitory concentrations above the clinical breakpoint. The frequency of these mutations remains low (<5% of isolates in most surveillance programs as of early 2024), but the selective advantage conferred in azithromycin-treated patients could drive rapid expansion under continued drug pressure.

8.2 Novel Therapeutic Approaches

Phage therapy the therapeutic use of bacteriophages to selectively kill bacterial pathogens has experienced a significant renaissance in the context of escalating antimicrobial resistance, including for typhoid fever. Multiple bacteriophages with specific lytic activity against *S. Typhi* have been characterized, and phage therapy has been explored in both preclinical models and compassionate use cases.^[64] The advantages of phage therapy for *S. Typhi* include high specificity that minimizes disruption of the gut microbiome, the ability of phages to self-amplify at sites of bacterial replication, and the theoretical capacity to engineer phages to deliver CRISPR-based genome-editing payloads that sensitize bacteria to conventional antibiotics. Challenges include the narrow host range of individual phages (requiring phage cocktails to address *S. Typhi* genetic diversity), phage resistance development, regulatory uncertainty, and limited manufacturing capacity for GMP-grade phage preparations.

Nanomedicine-based drug delivery systems represent another avenue of therapeutic innovation with potential relevance to typhoid fever. Nanoparticle encapsulation of conventional antibiotics has been explored as a strategy for overcoming multiple resistance mechanisms simultaneously: nanoparticle formulations can bypass efflux pump-mediated resistance by enabling direct intracellular delivery to infected macrophages, can achieve higher local concentrations within the SCV microenvironment where *S. Typhi* replicates, and can extend antibiotic half-life and reduce systemic toxicity.^[65] Chitosan nanoparticles loaded with ciprofloxacin have demonstrated significantly improved in vitro and in vivo activity against intracellular *S. Typhi* compared to free drug in macrophage infection models, with superior

uptake into the intracellular compartment and prolonged bactericidal activity.

Host-directed therapy (HDT) approaches that target host cellular processes required for *S. Typhi* intracellular survival represent a mechanistically distinct alternative to pathogen-targeted antibiotic therapy. Pharmacological inhibition of autophagy regulatory pathways, manipulation of macrophage activation states through cytokine modulators, and repurposed drugs including statins, metformin, and iron chelators that modulate the intracellular environment have been explored in preclinical studies.^[66] The theoretical advantages of HDT include reduced selective pressure for resistance (since the drug target is in the host rather than the pathogen) and potential synergism with conventional antibiotics when used in combination.

8.3 Personalized Medicine Approaches

The integration of pharmacogenomic information into typhoid treatment decisions represents an emerging frontier with particular relevance to patient populations where genetic polymorphisms significantly affect antibiotic pharmacokinetics and pharmacodynamics. Cytochrome P450 enzyme polymorphisms (particularly CYP1A2, which is involved in the metabolism of fluoroquinolones) and efflux transporter variants (including MDR1/ABCB1 polymorphisms affecting intracellular drug concentrations) have been shown to influence fluoroquinolone exposure and clinical response in several infectious disease contexts.^[67] Population-based data from South Asian and African populations—which carry the highest typhoid burden—suggest that clinically significant pharmacokinetic variability driven by pharmacogenomic factors may contribute to variable treatment outcomes observed in clinical practice.

9. VACCINATION AND PREVENTION STRATEGIES

9.1 Historical Development of Typhoid Vaccines

The history of typhoid vaccination spans nearly 130 years, beginning with the pioneering work of Almroth Wright and David Semple in the United Kingdom and Richard Pfeiffer and Wilhelm Kolle in Germany, who independently developed killed whole-cell typhoid vaccines in 1896–1897.^[68] These heat-killed, phenol-preserved whole-cell vaccines (WHOCVs) demonstrated significant efficacy in military populations and were widely deployed in the armed forces of several countries during World War I, contributing to a dramatic reduction in typhoid mortality compared to previous conflicts. However, they caused frequent and severe local and systemic reactogenicity—including fever, headache, and malaise severe enough to incapacitate recipients—which limited their acceptability for mass civilian immunization programs.

The development of the live attenuated oral Ty21a vaccine (Vivotif) in the 1970s represented a major advance in terms of tolerability. Ty21a, a chemically mutagenized *S.*

Typhi strain with multiple attenuating mutations affecting Vi antigen expression, galactose metabolism, and stress response, provides approximately 50–67% protection against laboratory-confirmed typhoid fever in field trials when given as a three- or four-dose oral course.^[69] Its oral administration obviates the need for injection equipment and trained healthcare workers for delivery. However, the requirement for multiple doses, refrigerated storage of liquid formulations, and the inability to use Ty21a in children under six years of age (due to concerns about inadequate immune response and residual virulence) limit its utility in the highest-burden populations.

9.2 Vi Polysaccharide Vaccines

The purified Vi polysaccharide vaccine (Vi-PS), licensed in the early 1990s under brand names including Typhim Vi (Sanofi Pasteur) and Typherix (GSK), provided a significant improvement in terms of the simplicity of the immunization regimen: a single intramuscular or subcutaneous injection provides approximately 55–72% protection against blood-culture-confirmed typhoid fever for 2–3 years in individuals over two years of age.^[70] The Vi-PS vaccine is well-tolerated, with predominantly mild local reactions and no serious adverse events, and can be co-administered with other vaccines. However, the T-cell-independent nature of the immune response to Vi polysaccharide—a characteristic of all unconjugated polysaccharide antigens—means that Vi-PS fails to elicit immunological memory, provides short-lived protection requiring repeat doses, and fails entirely to induce protective immunity in children under two years of age, who are among the most vulnerable to severe typhoid disease.

9.3 Typhoid Conjugate Vaccines

Typhoid conjugate vaccines (TCVs), in which the Vi polysaccharide is covalently linked to a carrier protein to convert the T-cell-independent polysaccharide response to a T-cell-dependent conjugate response, represent the most significant advance in typhoid vaccinology in the modern era. Three TCVs have received WHO prequalification: Typbar-TCV (Bharat Biotech International, India; Vi conjugated to tetanus toxoid), PedaTyph (Bio-Med, India; Vi conjugated to diphtheria toxoid CRM197), and ROTAVAX (PATH, development stage).^[71] The pivotal Mwangi *et al.* trial in Blantyre, Malawi, and a large randomized controlled trial in Kathmandu, Nepal, conducted by Shakya and colleagues, provided efficacy data demonstrating approximately 81.6% (95% CI 58.8–91.8%) and 79% (95% CI 69.5–85.3%) vaccine efficacy against blood-culture-confirmed typhoid fever, respectively, over 1.5–2 years of follow-up. Critically, TCVs elicit robust protective responses in infants and children under two years of age, filling the critical gap left by unconjugated Vi-PS vaccines.

The WHO Strategic Advisory Group of Experts on Immunization (SAGE) issued recommendations in 2017 and updated them in 2022 for the preferential use of

TCVs over older typhoid vaccines in routine immunization programs of endemic countries, specifically recommending vaccination at 9–15 months of age within the routine immunization schedule and catch-up vaccination through 15 years of age in programmatic rollouts.^[72] Countries including Pakistan, Zimbabwe, Liberia, and several others have subsequently introduced TCVs through their national immunization programs with support from Gavi, the Vaccine Alliance. The real-world effectiveness of Typbar-TCV in Pakistan documented in a prospective cohort study conducted during the XDR typhoid epidemic in Sindh was estimated at 84% against all typhoid fever and 87% against XDR typhoid, demonstrating that TCV confers substantial protection even against highly resistant strains.

9.4 Vaccine Implementation Challenges

Despite the compelling evidence of TCV efficacy and the strong WHO recommendation for their use, significant challenges remain in achieving equitable access to these vaccines in the highest-burden populations. Cost remains a major barrier: even at Gavi-supported prices of approximately US\$1.50–2.00 per dose for eligible LMICs, the total programmatic cost of TCV introduction including cold chain infrastructure, delivery, and

communications—places a substantial demand on already-stretched national immunization budgets.^[73] Middle-income countries not eligible for Gavi support face market prices significantly higher than subsidized Gavi prices, creating a perverse situation in which countries with substantial typhoid burden but higher income classification face the greatest financial barriers to TCV introduction.

Cold chain requirements represent a practical implementation challenge that disproportionately affects health systems in remote or under-resourced settings. Current TCV formulations require storage at 2–8°C throughout the supply chain, necessitating reliable electricity supply and functioning cold chain equipment at all levels from national warehouse to service delivery point.^[74] Controlled Temperature Chain (CTC) approaches, which allow vaccines to be stored at ambient temperatures for limited periods under documented conditions prior to administration, are being explored for TCVs but have not yet been licensed for these products. The development of thermostable TCV formulations would significantly expand the reach of vaccination programs in settings where cold chain infrastructure is unreliable.

Table 3: Comparison of Licensed Typhoid Vaccines.

Vaccine	Type	Dose Schedule	Minimum Age	Efficacy	Duration	WHO Recommendation
WHOCVs	Killed whole-cell	2 doses SC/IM	2 years	51–67%	3 years	No longer recommended
Ty21a (Vivotif)	Live attenuated oral	3–4 oral doses	6 years	50–67%	5–7 years	Alternative option
Vi-PS (Typhim Vi)	Vi polysaccharide	1 dose IM	2 years	55–72%	2–3 years	Acceptable if TCV unavailable
Typbar-TCV	Vi-TT conjugate	1 dose IM	6 months	79–82%	>5 years (est.)	Preferred (WHO prequalified)
PedaTyph	Vi-CRM197 conjugate	1 dose IM	3 months	~80%*	>5 years (est.)	Preferred (WHO prequalified)

*Immunogenicity-based estimate; phase III efficacy trial ongoing. SC = Subcutaneous; IM = Intramuscular; Vi-TT = Vi-Tetanus Toxoid; Vi-CRM197 = Vi-non-toxic CRM197 diphtheria carrier protein.

9.5 WASH Interventions and Integrated Prevention

Water, sanitation, and hygiene (WASH) improvements represent the most fundamental and sustainable approach to typhoid control, addressing the environmental transmission pathways that sustain the disease in endemic settings. Systematic reviews of the evidence base for WASH interventions against typhoid fever have consistently demonstrated significant protective effects: access to piped, treated water supply is associated with approximately 80% reduction in typhoid incidence compared to unimproved water sources; improved sanitation (use of hygienic toilets that safely contain excreta) is associated with 29–67% reduction in incidence; and hand hygiene promotion programs have demonstrated 30–50% reductions in enteric disease outcomes including typhoid.^[75]

Mathematical modeling analyses have explored the comparative cost-effectiveness of vaccination versus WASH interventions for typhoid control and have generally found that both approaches are cost-effective in high-burden settings but that they operate through complementary mechanisms and generate additive benefits when implemented together. Integrated prevention strategies that combine vaccination campaigns with WASH infrastructure investment and hygiene behavior change communication are increasingly recognized as the most rational approach to sustained typhoid control, particularly in rapidly urbanizing settings where short-term vaccination provides immediate protection while longer-term WASH improvements progressively reduce transmission and the need for repeated vaccination campaigns.^[76]

10. GENOMICS, ARTIFICIAL INTELLIGENCE, AND FUTURE RESEARCH DIRECTIONS

10.1 Whole-Genome Sequencing and Phylogeography

Whole-genome sequencing (WGS) has transformed our understanding of *S. Typhi* population structure, evolutionary dynamics, and global transmission patterns in ways that were unimaginable with earlier genotyping approaches. The comprehensive phylogenetic framework established by Pathogenwatch and the Global Typhoid Genomics Consortium, based on the analysis of thousands of *S. Typhi* genomes from diverse geographic settings and time periods, has revealed a deep genomic diversity organized into four primary clades (designated 0, 1, 2, 3, and 4) and multiple sub-lineages with distinct geographic distributions and resistance profiles.^[77] The H58 lineage (now designated 4.3.1 in the updated Pathogenwatch nomenclature), which encompasses virtually all MDR and XDR isolates, has demonstrated rapid global expansion over the past three decades and now constitutes the dominant lineage circulating in South and Southeast Asia.

WGS-based surveillance has the capacity to track the real-time evolution and spread of resistance determinants in *S. Typhi* populations and to detect the emergence of novel resistance mechanisms before they become epidemiologically dominant.^[78] The integration of genomic data with epidemiological metadata—including patient travel history, residence location, source of water and food—enables reconstruction of transmission chains and identification of previously unrecognized outbreak clusters. Several national reference laboratories and international genomic surveillance networks, including the Wellcome Sanger Institute's Pathogen Informatics group and the US CDC's National Enteric Disease Surveillance program, have implemented routine WGS for *S. Typhi* reference isolates, creating valuable longitudinal genomic surveillance resources.

10.2 Artificial Intelligence Applications in Typhoid Research

Artificial intelligence (AI) and machine learning (ML) methodologies are being applied across multiple dimensions of typhoid fever research and surveillance. In the domain of outbreak prediction, deep learning models trained on historical case notification data, climate variables, population density indices, and WASH infrastructure quality assessments have been developed and validated for forecasting typhoid fever incidence at sub-national spatial scales in endemic countries.^[79] Such predictive tools have the potential to inform the timing and geographic targeting of vaccination campaigns and WASH interventions, directing resources to populations at highest risk during predicted epidemic periods.

In diagnostics, ML algorithms have been applied to clinical and laboratory data to develop decision-support tools for typhoid diagnosis in resource-limited settings where definitive microbiological testing is unavailable.

Random forest and gradient boosting models trained on clinical features, basic laboratory parameters, and demographic characteristics have demonstrated area-under-ROC-curve values of 0.82–0.91 for distinguishing typhoid fever from other febrile illnesses in endemic settings—performance comparable to or exceeding that of available rapid serological tests [80]. These algorithms, if validated prospectively and implemented in accessible digital formats (smartphone applications, tablet-based decision tools), could significantly improve the accuracy of empirical typhoid diagnosis at primary care level.

10.3 Identified Research Gaps

Despite substantial advances, the typhoid fever research field retains important knowledge gaps that must be addressed to achieve the goal of sustainable disease control. First, the immunological correlates of protection against typhoid fever remain incompletely characterized. While Vi-specific serum IgG concentrations have been used as a surrogate marker of vaccine immunogenicity, the threshold concentration associated with protection has not been definitively established, and the contributions of mucosal IgA, *S. Typhi*-specific CD4⁺ T cell responses, and innate immune memory to overall protective immunity are poorly understood.^[81] Establishing validated immunological correlates of protection would accelerate vaccine development by enabling immunological bridging strategies for establishing vaccine efficacy without large field trials.

Second, the long-term durability of TCV-induced immunity requires further investigation. Phase III trials of Typhbar-TCV and other TCVs have thus far provided efficacy data over maximum follow-up periods of 2–3 years; the trajectory of protective immunity over 5–10 years and the potential need for booster doses in high-transmission settings are unknown.^[82] This uncertainty complicates the cost-effectiveness analyses needed to guide national immunization program decision-making. Third, the potential for vaccine escape—*S. Typhi* strains that have undergone mutations in the Vi capsule biosynthesis or regulatory genes that could enable evasion of TCV-induced immunity—while not yet documented as a public health concern, deserves proactive monitoring given the precedent from other encapsulated bacteria.

11. PUBLIC HEALTH POLICIES AND GLOBAL INITIATIVES

11.1 WHO Strategies and International Programs

The World Health Organization's Immunization Agenda 2030 (IA2030) explicitly identifies typhoid conjugate vaccines as a priority product for introduction in endemic LMICs, with a target of achieving at least 80% coverage in the highest-burden countries by 2030.^[83] WHO's Initiative for Vaccine Research (IVR) has supported the development of improved typhoid vaccine candidates including bivalent typhoid/paratyphoid A vaccines, live attenuated CVD 909 strains, and recombinant protein

vaccines targeting multiple *S. Typhi* antigens beyond Vi. The Gavi Alliance has made available US\$85 million for TCV introduction support through its vaccine investment strategy, enabling co-financing of TCV programs in 50 lower-income countries eligible for Gavi support.

The WHO Global Antimicrobial Resistance Surveillance System (GLASS), launched in 2015 and incorporating enteric fever pathogens from its inception, has strengthened systematic collection of antimicrobial susceptibility testing data from national reference laboratories in endemic countries and enabled the detection and early warning of emerging resistance patterns including XDR typhoid.^[84] The GLASS network has enrolled over 70 member countries, though significant gaps remain in coverage of the highest-burden regions of South Asia and sub-Saharan Africa, where laboratory capacity constraints limit the representativeness and timeliness of surveillance data.

11.2 National Control Strategies: Case Studies

Pakistan's experience with XDR typhoid control provides instructive lessons for other endemic countries. Following the declaration of the XDR typhoid epidemic in Sindh Province in 2016, the Government of Pakistan, with support from WHO, UNICEF, and international partners, implemented a comprehensive response strategy combining emergency vaccination campaigns with Typbar-TCV (targeting children 6 months–15 years in highest-burden districts), enhanced surveillance strengthening, laboratory capacity building, and public health communication.^[85] The vaccination campaign achieved high coverage (>85% in targeted age groups) in focused geographic areas and was associated with a significant reduction in XDR typhoid incidence in vaccinated populations relative to unvaccinated comparison groups. Pakistan subsequently became the first country to introduce TCV into its routine national immunization program in late 2019 with Gavi support—a landmark achievement that has since been followed by several other countries.

Zimbabwe's TCV introduction campaign, implemented in 2019 with Gavi support following recurring typhoid outbreaks in Harare and other cities, provides another important case study. Zimbabwe conducted a nationwide school-based vaccination campaign targeting children 9 months–15 years, achieving coverage estimates of 83% in the target age group.^[86] Preliminary surveillance data suggest a significant decline in laboratory-confirmed typhoid cases following the campaign, though definitive vaccine effectiveness estimates are being refined through ongoing prospective studies. The Zimbabwe experience highlights both the feasibility of large-scale TCV campaigns in sub-Saharan African settings with limited health system capacity and the challenges of sustaining gains through integration into routine immunization programs post-campaign.

11.3 One Health Perspectives

While *S. Typhi* is a strictly human-restricted pathogen—in contrast to non-typhoidal *Salmonella* serovars, which have extensive animal and environmental reservoirs—a One Health perspective that integrates human, animal, and environmental health frameworks remains relevant to typhoid control for several reasons.^[87] The contamination of drinking water sources, irrigation water, and food with human fecal material the proximate source of *S. Typhi* for all non-carrier-related infections is intimately connected to land use patterns, agricultural practices, and ecosystem integrity. Wastewater irrigation of vegetables, a practice widespread in peri-urban settings of endemic countries in the absence of adequate sanitation infrastructure, represents a particularly important interface between environmental management and enteric disease transmission.

The One Health framework also highlights the importance of food safety systems in typhoid control. Street food—a major source of nutrition for urban poor populations in South Asia and Africa has been implicated in multiple typhoid outbreak investigations as a vehicle for *S. Typhi* transmission, reflecting inadequate food safety practices in informal food vending contexts.^[88] Regulatory strengthening of food safety standards, combined with training and infrastructure support for street food vendors regarding safe water use, handwashing facilities, and temperature management, represents an underexplored but potentially high-impact intervention for typhoid control in urban settings.

12. CRITICAL ANALYSIS AND DISCUSSION

12.1 Synthesis of Evidence

The literature synthesized in this review converges on several overarching conclusions about the current state of typhoid fever research and control. First, the disease burden remains enormous and substantially underappreciated: systematic reviews and modelling exercises consistently identify a several-fold gap between reported and estimated true case counts, reflecting the inadequacy of passive surveillance in endemic settings.^[89] This evidence gap has real policy consequences, as the economic and public health burden of typhoid fever continues to be deprioritized relative to diseases with more visible surveillance infrastructure, perpetuating a cycle of underinvestment in control measures.

Second, the emergence of XDR typhoid has created a genuine therapeutic emergency that demands urgent response on multiple fronts simultaneously: rapid expansion of TCV coverage to reduce transmission and disease burden; antibiotic stewardship programs to preserve the efficacy of remaining treatment options; and accelerated development of novel antimicrobial agents active against resistant *S. Typhi*. The concentration of XDR typhoid in Pakistan, while representing a public health crisis for that country, also provides a unique opportunity for evaluating the impact of large-scale TCV

implementation in a setting with documented XDR circulation.^[90]

Third, the diagnostic landscape for typhoid fever remains inadequate relative to the clinical need. The continued widespread use of the Widal test despite decades of evidence demonstrating its unreliability—reflects the absence of affordable, accurate alternatives deployable at primary care level in endemic settings. Significant investment in point-of-care molecular diagnostics and their clinical validation in representative endemic settings is urgently needed to bridge this gap.^[91] Fourth, the convergence of genomic surveillance, mathematical modeling, and implementation science is creating new opportunities for targeted, evidence-based control strategies that can be adapted to the specific epidemiological context of different endemic settings.

12.2 Strengths and Limitations of Current Research

The research base synthesized in this review has several important strengths. The availability of large-scale multi-site epidemiological studies (GEMS, SEAP, SETA) conducted with rigorous methodology in multiple endemic countries has substantially improved the quality of disease burden estimates compared to earlier single-site studies. The development of the Global Typhoid Genomics Consortium database has created an unprecedented resource for phylogeographic and resistance surveillance research. The completion of randomized controlled trials of TCVs meeting WHO-prequalification standards of evidence for efficacy, safety, and immunogenicity has provided a solid foundation for vaccination policy recommendations.^[92]

Against these strengths must be weighed significant limitations. Geographic concentration of research capacity in a small number of academic institutions and reference laboratories in high-income countries and a few regional centers in endemic countries creates asymmetries in research agenda-setting that may not adequately reflect the priorities of the communities most affected by typhoid fever. The majority of randomized controlled trials of typhoid vaccines have been conducted in South Asia, with limited data from sub-Saharan Africa on real-world vaccine effectiveness in different transmission and malnutrition contexts. The evidence base for novel therapeutics including phage therapy and HDT approaches is almost entirely preclinical or based on case reports, reflecting the early stage of development of these modalities.^[93]

12.3 Knowledge Gaps and Future Research Priorities

On the basis of this comprehensive review, we identify the following as the highest-priority research gaps requiring urgent attention. In the area of vaccines, longitudinal immunogenicity and effectiveness studies of TCVs extending beyond five years post-immunization are needed to establish booster dose schedules; head-to-head comparative immunogenicity trials of different TCV products are needed to identify the most immunogenic

formulations; and next-generation multi-antigen vaccines that combine Vi polysaccharide with additional *S. Typhi* antigens (flagellin, outer membrane proteins, hemolysin E) to broaden protective immunity and potentially address variant strains should be prioritized.^[94]

In diagnostics, there is an urgent need for prospective clinical validation of CRISPR-based and other novel diagnostic platforms using well-characterized clinical specimen biobanks from endemic settings; for the development and evaluation of clinical diagnostic algorithms combining multiple biomarkers for typhoid diagnosis; and for implementation research on the optimal deployment strategies for point-of-care diagnostics within primary healthcare systems in LMICs. In therapeutics, early-phase clinical trials of phage therapy and HDT approaches in typhoid fever are needed; the therapeutic potential of newly identified antimicrobial compound classes active against *S. Typhi* (including teixobactin, odilorhabdins, and novel fluoroquinolone analogues) requires evaluation; and azithromycin resistance surveillance must be intensified and standardized globally.^[95]

13. CONCLUSION

Typhoid fever occupies a paradoxical position in global health: it is a disease caused by a strictly human-restricted pathogen with a well-characterized biology, an available preventive vaccine, and a theoretical pathway to elimination yet it continues to claim tens of thousands of lives annually and sicken millions more, predominantly in communities that lack access to the most basic public health infrastructures of safe water and sanitation. The clinical and public health challenges of typhoid fever in the 21st century are qualitatively different from those of previous eras, shaped by the emergence of XDR strains that have stripped away most of the antibiotic armamentarium, by the availability of highly effective conjugate vaccines whose implementation remains constrained by financial and logistical barriers, and by the growing recognition that surveillance systems have systematically underestimated the true disease burden.

This review has synthesized evidence across the full spectrum of typhoid fever research—from the molecular biology of *S. Typhi* pathogenesis to the global epidemiology of antimicrobial resistance, from the evaluation of diagnostic technologies to the implementation science of vaccination programs to provide a current, comprehensive, and critically analyzed assessment of the field. Several overarching conclusions emerge. The XDR typhoid epidemic originating in Pakistan represents a sentinel event that demands an urgent, sustained, and internationally coordinated response; failure to act decisively risks the further evolution and global spread of strains against which current therapeutic options are severely limited. Typhoid conjugate vaccines are highly effective, safe, and suitable for integration into routine immunization programs, and

their equitable global rollout must be accelerated with commensurate financial investment from donor governments and international institutions.

The inadequacy of current diagnostic tools particularly in primary care settings in endemic countries where most typhoid fever goes undiagnosed or misdiagnosed perpetuates cycles of inappropriate antibiotic use that accelerate resistance evolution, delayed treatment that drives severe complications, and unreliable surveillance data that undermine the case for prevention investments. Developing and deploying affordable, accurate point-of-care diagnostics must be recognized as a global health priority comparable in urgency to vaccine development. Simultaneously, the long-term solution to typhoid fever lies in the elimination of the environmental conditions that sustain transmission: universal access to safely managed water and sanitation facilities remains the most fundamental and durable intervention against this disease of poverty.

Looking to the future, the convergence of genomic surveillance technologies, mathematical modeling frameworks, artificial intelligence-assisted diagnostic tools, and novel therapeutic platforms creates genuine grounds for optimism that the technical arsenal available for typhoid control will continue to improve. Translating these technical advances into health impact for the populations most burdened by typhoid fever will require not only scientific innovation but also sustained political commitment, equitable financing mechanisms, strengthened health system capacity in endemic countries, and a global commitment to addressing the structural determinants of poverty and inadequate infrastructure that make typhoid fever a disease of the 21st century. The goal of a typhoid-free world is achievable within a generation but only if the evidence synthesized in this review is translated into sustained, adequately resourced, and equitably implemented action.

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