Typhoid fever

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Competing interests

The authors declare no competing interests.

Abstract

Typhoid fever is an invasive disease associated with bloodstream infection causing a high burden of disease in Africa and Asia, where it primarily affects individuals ranging from infancy through to young adulthood. The causative organism, *Salmonella enterica* serovar Typhi is transmitted via the faecal-oral route, crossing the intestinal epithelium and disseminating to systemic and intracellular sites, causing an undifferentiated febrile illness. Blood culture remains the practical reference standard for diagnosis of typhoid fever, where this is available, but newer diagnostic modalities are an important priority under investigation. There has been remarkable progress since 2017 in defining the twin global burdens of typhoid disease and antimicrobial resistance; in understanding disease pathogenesis and immunological protection through the use of controlled human infection; and in advancing effective vaccination programmes through strategic multi-partner collaboration and targeted clinical trials in multiple high-incidence priority settings. This primer article thus offers a timely update of progress and perspective on future priorities for the global scientific community.

[H1] Introduction

Typhoid fever is a serious invasive infection involving the blood-stream and deep reticulo-endothelial tissues. The organism responsible for the clinical syndrome of typhoid fever, *Salmonella enterica* (*S. enterica*) subspecies *enterica* serovar Typhi (*S.* Typhi), is found within the Enterobacterales family. It is a rod-shaped, Gram-negative, facultative anaerobic bacteria within the *Salmonella* genus, and is host-restricted to humans.[1]

The World Health Organisation (WHO) defines a confirmed case of typhoid fever as an individual with laboratory confirmation by culture or molecular methods (such as detection of *S.* Typhi DNA) of *S.* Typhi from a normally sterile site. A suspected case is defined as an individual with fever for at least three out of seven consecutive days in an endemic area or following travel from an endemic area, or being a household contact of a confirmed case.[2]

In endemic areas without access to appropriate diagnostics, clinical diagnosis is relied upon. However, with numerous other infectious conditions presenting with a similar undifferentiated fever this lacks both sensitivity and specificity.[3] Typhoid was the first human disease in which healthy asymptomatic carriage was demonstrated, in 1904, to be a source of disease transmission,[4] including in the famous case of Mary Mallon.[5]

Approximately 2-5% of cases of acute typhoid illnesses have historically been thought to develop asymptomatic chronic carriage.[6] This is defined as apparently healthy individuals with evidence of shedding of *S.* Typhi in stool at least 12 months after finishing an appropriate course of antimicrobial treatment and the resolution of symptoms, following a laboratory confirmed episode of acute disease, or alternatively, two positive stool samples for *S.* Typhi 12 months apart.

S. Typhi is transmitted via the faecal-oral route crossing the intestinal epithelium and disseminating to systemic sites. Blood culture remains the practical reference standard for diagnosis of typhoid fever, where available. Timely administration of appropriate antimicrobials are the mainstay of treatment for typhoid fever, but with escalating antimicrobial resistance this is becoming challenging in some parts of the world.

With improvements in sanitation infrastructure, drinking water quality, and food safety typhoid fever can be controlled. However, in some low-resource settings the comprehensive changes required in such infrastructure may take decades or even generations, and the burden of disease from infancy through to young adulthood, remains unacceptably high.

The term “enteric fever” also encompasses the clinical syndrome caused by *Salmonella enterica* serovars Paratyphi A, B, or C (*S.* Paratyphi A, B, C). A full description of paratyphoid fever is beyond the scope of this primer, but it will be mentioned in brief where there are relevancies, similarities, or contrasts – in particular for *S*. Paratyphi A, which accounts for around a quarter of enteric fever cases in South Asia, but is currently much rarer in Africa.[7] *Salmonella* serovars other than *S.* Typhi and *S.* Paratyphi A, B, or C are known as non-typhoidal *Salmonella* (NTS). Consideration of NTS disease is also beyond the scope of this Primer.

In this Primer article we will discuss the epidemiology of typhoid fever, detailing the burden and pattern of disease, modes of transmission, and risk factors for infection. We will explore the literature on *S.* Typhi bacterial genomics as well as pathogenesis and the host response to infection. With the rising problem of global antimicrobial resistance (AMR), we will outline the current patterns of resistance throughout the world and the antimicrobial treatment options available. Since typhoid has a variable and often non-specific clinical presentation, improved diagnostics for clinical and epidemiological use are essential, and we explore this.

[H1] Epidemiology

[H2] Reservoir, source, and mode of transmission

S. Typhi is a human-restricted pathogen with no non-human animal reservoirs.[8] *S.* Typhi is shed in human faeces from sites of infection in the gallbladder and small bowel. The major source of new infections in high-incidence areas with poor sanitation infrastructure is indirect transmission via water and also via food contaminated with the faeces[9] of an infected person, who may shed the bacteria during acute infection, convalescence, or chronic carriage. As typhoid fever incidence declines, the treatment of chronic carriers with antimicrobials and sometimes cholecystectomy may become important. Direct transmission associated with oral-anal sex has also been reported.[10] In addition, *S.* Typhi may also survive outside the human host for extended periods without evidence of multiplication,[11] in a viable, non-culturable state, contributing to persistence and transmission over large

distances and longer time scales.[12] Changes in expression of *S.* Typhi genes linked to central metabolism, stress associated with arrested proton motive force, and respiratory chain factors may provide insights into the mechanisms for survival of *S.* Typhi in aqueous and other environments.[13] Improvements in the sensitivity of detection of *S.* Typhi in environmental samples by nucleic acid amplification have led to progress with investigating environmental contamination as a means to understand community-level risk of typhoid fever.[14]

[H2] Measuring Disease burden

S. Typhi is a leading cause of community-onset bloodstream infection in studies from countries in south and southeast Asia,[15] and an important but often less prominent cause in studies from countries in Africa.[16,17] The number and geographic representativeness of enteric fever and typhoid fever incidence and outcome studies has improved greatly over the past two decades,[18-22] as have approaches to extrapolating incidence,[23-26] and modelling burden of disease.[27]

Typhoid fever was estimated to cause 10.9 (95% uncertainty interval, UI 9.3–12.6) million illnesses globally, and 116,800 (95% UI 65,400–187,700) deaths globally in 2017[27] The global case fatality ratio is estimated at 0.95%, but may be higher among the very young and the elderly. A global incidence model for this estimate (based on both population-based cohorts and national surveillance data in medium and high-incidence regions, with additional use of vital registration sources in low-burden regions) estimated a global incidence of enteric fever of 197·8 (172·0–226·2) per 100 000 person-years.[7]

Considering variation by super-regions, south Asia had the highest age-standardised incidence rate of enteric fever (549 [481–625] cases per 100 000 person-years) and the largest number of illnesses (10·3 million [9·0–11·7]), accounting for 71·8% of global illnesses in 2017. Southeast Asia, east Asia, and Oceania combined accounted for 14·1% of global illnesses (2·02 million [1·82–2·23]) with an incidence ranging from 51.0 (east Asia) to 219.8 (southeast Asia) cases per 100,000 person-years. Sub-Saharan Africa accounted for 12·1% of enteric fever cases (1·73 million [1·45–2·06]), and had an incidence ranging by from 161 and 151 cases per 100,000 person-years in West and East Africa respectively, to 2.3 per 100,000 person-years in southern Africa.[7]

Since there is no syndromic 'envelope' of disability adjusted life years for febrile illnesses such as typhoid fever, a 'natural history' approach is taken to estimate burden of disease.[28] For this, studies of typhoid incidence using active population-based surveillance or hybrid surveillance are collated[29,30] and extrapolated to areas without data, and the prevalence of major complications such as intestinal perforation and the case fatality ratio[31,32] are applied to estimate disability and death due to typhoid fever.[27] Overall, enteric fever was responsible for 8.4M (4.7 - 13.6) disability-adjusted life years in 2017, comprising 8.3M (4.6 - 13.4) years of life lost and 105,000 years lived with disability).[7] The frequency and clinical presentations of severe and complicated disease are discussed below.

[H2] Risk factors

[H3] Age

In high- and medium-incidence endemic settings, disease is seen from infancy onwards. Although globally the overall peak age of disease is at 5-9 years, this average conceals considerable heterogeneity between regions and countries.[7] The peaks and decline in incidence of typhoid fever with age in endemic settings are considered to be related to the local force of infection leading to immunity gradually and cumulatively acquired from natural infection and repeated subclinical or asymptomatic exposure to the pathogen.[33] This means that acoss these age-bands there is considerable variation in age-distribution by location. Incidence may be high or even peak among infants in very high incidence areas, and may peak later, among older children or even young adults, in areas of medium incidence. Incidence subsequently declines gradually with age through adulthood, and incidence is typically low in all elderly populations.[27]Re-infection, as opposed to relapse, has been documented in the historical and more recent literature with levels of protection conferred by an episode of clinical infection thought to be only moderate.[34]

[H3] Environmental exposures. A recent systematic review and meta-analysis of case-control studies evaluated associations between typhoid fever and water, sanitation, and hygiene (WASH) and food exposures.[35] The authors identified 19 manuscripts describing 22 case-control studies, with 20 (90.9%) having medium or high risk of bias. In the meta-

analysis, good hygiene and water treatment were most strongly associated with protection from typhoid fever (OR = 0.52 and 0.59, respectively), whereas poor hygiene and untreated water were most strongly associated with the risk of typhoid fever (OR = 2.2 and 2.4, respectively). Of three sanitation factors, only unsafe waste management was significantly associated with typhoid fever (OR = 1.6, 95% CI = 1.3–2.0). Protective food practices were significantly associated with lower odds of typhoid fever (OR = 0.74), and risky food practices and consuming food or drink outside the home were associated with significantly higher odds of typhoid fever (OR = 1.6–1.7). Dairy, ice cream, and fruits and juices were significantly associated with typhoid fever (OR = 1.4–1.5).[35] In a cluster randomized controlled trial of typhoid conjugate vaccine (TCV), living in a household with better WASH practices at baseline was independently associated with a significant reduction in the odds of typhoid fever.[36]

In contrast, in typhoid non-endemic countries, cases of typhoid fever are almost exclusively related to recent travel, contact with a traveller from an endemic country, or with food preparation by a chronic carrier.[37]

[H3] Human genetic factors.

A genome-wide association study performed among individuals with and without blood culture-confirmed enteric fever in Vietnam showed a strong association with higher risk of infection at rs7765379, a marker mapping to the HLA class II region, in proximity to HLA-DQB1 and HLA-DRB1.[38] The finding was replicated in a large cohort in Nepal, and in a second independent collection from Vietnam. HLA-DRB1 was implicated as a major contributor to resistance against enteric fever, likely mediated by antigen presentation.

[H3] *Seasonal and environmental factors*

Improvements in WASH and food exposures, and increased use of TCV in typhoid-endemic countries, are likely to strengthen typhoid fever prevention and control. An analysis of seasonal patterns of typhoid and paratyphoid fevers observed a distinct seasonal pattern by latitude, with seasonal variability more pronounced further from the equator.[39] The investigators found evidence of a positive association between preceding rainfall and enteric fever among settings 35°–11°N and a more consistent positive association between

higher temperature and enteric fever incidence across most regions of the world. The underlying mechanisms that drive the seasonality of typhoid fever are poorly understood. It is likely that the impacts of climate change that contribute to faecal contamination of water and food, such as flooding, water shortages that increase dependence on unsafe water, and deterioration in food safety may be anticipated to increase the risk for typhoid fever.[40-42]

[H2] Pathogenic variants

Distinct variants of *S.* Typhi and *S.* Paratyphi A have been recognised for over a century using phage typing,[43] but this approach has now been replaced by pathogen genome sequencing.[44] Global diversity studies have shown that both pathogens harbour multiple distinct phylogenetic lineages, which are associated with specific geographic regions.[45,46] There is no evidence that different *S.* Typhi or *S.* Paratyphi A variants are associated with demographic factors such as age or sex,[47,48] and they do not appear to be associated with with differing disease presentations or severity, but rather the distribution of variants appears to be primarily geographically determined. *S.* Typhi variants are defined and identified using the GenoTyphi genotyping scheme, which was first developed in 2016 using nearly 2,000 pathogen genome sequences from 65 countries.[49] The scheme is regularly updated to reflect newly identified variants or genotypes, as pathogen sequencing becomes more widespread.[50] The latest updates to the scheme (December 2022) were based on the analysis of 13,000 genomes from 111 countries by the Global Typhoid Genomics Consortium.[51] These data provide a comprehensive view of the distribution of *S.* Typhi variants across countries and regions, although some regions, especially Central and Northern Africa, Western Asia and Latin America, still lack sequence data. The distribution of variants is quite distinct by region (see **Figure 2**).[51] For example, genotype 4.3.1 (previously known as H58) dominates the pathogen population in South Asia (where it is thought to have emerged in the early 1990s)[52] and Eastern Africa (where it is thought to have been introduced multiple times in the last 10-20 years),[47] but is rare elsewhere. In Western Africa, the dominant genotypes are 3.1.1 and 2.3.1,[53] whereas Central and South America have distinct variants (2, 2.5, 3.5),[54,55] and island nations have their own genotypes (3.5 in Samoa, 3.5; 4.2 in Fiji, 4.2; 2.1.7 in Papua New Guinea).[50,56,57] The reason for geographic separation of variants is not fully understood, but it is proposed that it is driven by human migration patterns since *S.* Typhi is a human-restricted pathogen.[58] For example,

the transfer of 4.3.1 to Eastern Africa could be linked to frequent migration of South Asians to Kenya and neighbouring countries in East Africa, whereas the distinct *S.* Typhi populations in Western Africa could reflect the stability of communities within these settings.

[H1] Mechanisms/Pathophysiology

While non-typhoidal *Salmonella enterica (S. enterica)* serovars cause foodborne gut luminal inflammation and enterocolitis in healthy humans, *S.* Typhi once ingested can rapidly cross the intestinal epithelium and disseminate to systemic sites, including the liver, spleen, bone marrow, and gallbladder **(Figure 3)**. *S*. Typhi is unusual among *S. enterica* serovars in that it harbours an exopolysaccharide capsule known as Vi which is the target of modern conjugate vaccines.[59] The Vi capsule is thought to be essential to *S.* Typhi pathogenesis; however, *S.* Paratyphi A causes a clinically indistinguishable infection despite lacking a Vi capsule, and these 2 human-adapted invasive serovars do not share any additional or unique virulence factors. When compared to non-typhoidal serovars which have a broader host-range among vertebrates, however, the genomes of serovars Typhi and Paratyphi A show evidence of functional gene loss, which is characteristic of host-restricted adaptation. Approximately 4% of *S.* Typhi and *S.* Paratyphi A genes carry these inactivating mutations, known as pseudogenes, compared with ≤1% in other non-typhoidal *S. enterica* serovars.[60-63]

[H2] Insights from *in vivo* and *ex vivo* disease models

[H3] Infection of intestinal epithelium and dissemination to tissues

Due to the human-restricted nature of *S.* Typhi and *S.* Paratyphi A, much of the foundational understanding of typhoid pathogenesis has been from the study of infection of susceptible mice with *S. enterica* serovar Typhimurium (*S.* Typhimurium), and a range of related *in vivo* and *ex vivo* models which have yielded many mechanistic insights into the complex interplay between pathogen adaptations, the microbiota and the host response.[63] Following oral ingestion by mice, generalist non-typhoidal serovars such as *S.* Typhimurium survive gastric acidity and evade colonisation resistance through inducing inflammatory competition with the resident microflora, thus altering the metabolic landscape in the lumen to optimise

access to luminal host-derived resources such as oxygen, nitrate, tetrathionate, and lactate.[64] *Salmonella* Typhi , in contrast, is a "stealth" pathogen that has several adaptation to rapidly cross the gut epithelium, inducing minimal imflammation[65,66]. Invasive strains of Salmonella possess the TviA regulatory locus, coding a protein with a complex counter-balanced regulatory function, downregulating flagellin-associated inflammation and regulating expression of the Vi capsule polysaccharide which mediates immune evasion.[67] The genes encoding the Vi capsule comprise the *viaB* locus within *Salmonella* pathogenicity island (SPI)-7, which also encodes the type III invasion-secretion system (T3SS) effector, SopE and a type IVB pilus.[68]

Salmonella cross the intestinal barrier by a multiplicity of routes. These include by direct invasion of enterocytes, invasion by a transcellular route, by direct uptake by dendritic cells across the epithelium, or by Salmonella invasion of specialised antigen-sampling epithelial M-cells (microfold cells) which overlie the organised lymphoid tissue of Peyer's patches, found particularly in the terminal ileum.[69] Salmonellae are transported through M cells to be presented to B-cells and dendritic cells that sit within the M-cell microfolds in Peyers patches.[70] Chronic infection of the lymphoid tissue in human intestinal Peyers patches is a key element of pathogenesis, acting as a source of ongoing enteric shedding in the stool and transmission, and may also lead to necrosis, and the serious disease complication of intestinal perforation.

Once Salmonellae have gained access to the host circulation, they can disseminate by several stealth mechanisms.[71] During extracellular vascular dissemination, the Vi capsule inhibits phagocytosis and confers serum resistance, likely by shielding the surface lipopolysaccharide O-antigen from antibodies.[72] However, the ability to survive and disseminate intracellularly is a key pathogenic strategy, and bacteria are also translocated from the gut within CD18 expressing phagocytes. This cellular population encompasses the reticulo-endothelial system (RES) an includes monocyte/macrophages, dendritic cells, polymorphonuclear leukocytes, and phagocytes in the liver, spleen, and bone marrow. Within minutes of contact with cells, invasive *Salmonella* are internalized in the *Salmonella*-containing vacuole,[60] a highly specialised modified phagosome that has the important function of evading endosomal fusion with the phagocyte oxidase complex, thus establishing a chronic, deep-seated intracellular reticuloendothelial infection.[65]

It is this established infection that permits Salmonellae to finally enter and colonise the gall bladder, an important niche from where they may be shed back into the gastrointestinal tract in bile – the hallmark and mechanism of chronic carriage of typhoid in human disease, enabling onward transmission of the pathogen to new hosts.

[H2] Controlled human infection model (CHIM)

To further our understanding of human typhoid pathogenesis and accelerate development of candidate vaccines, a controlled human infection model (CHIM) for study of typhoid infection, based on an earlier model from the University of Maryland in the 1950s, was established at Oxford University in 2011.[73] The model involves the deliberate infection of healthy adult volunteers with an antibiotic-sensitive strain of *S.* Typhi, manufactured under Good Manufacturing Practice (GMP), originally derived from the gallbladder of a woman with chronic typhoid infection in Maryland in the 1950s.[73,74] After screening and informed consent procedures, participants ingested 10,000 CFU of *S.* Typhi in a bicarbonate solution. Approximately two thirds of individuals developed a fever for $\geq$12 hours and/or bacteraemia over the next 2 weeks (median time to onset 8 days), thus meeting the study definition of typhoid fever and triggering cessation of infection with oral antibiotics.[73] A similar model was established to study paratyphoid infection, though 1,000 CFU of *S*. Paratyphi A were found to be sufficient for consistent infection (60%).[75] In the paratyphoid model the proportion of individuals with bacteraemia, and the cytokine responses of participants were similar to those in the typhoid model but bacteraemia was more prolonged (median 53 hours), and blood-culture positive asymptomatic infection was more common (55%).[75]

[H3] Inflammatory response

The typhoid model showed evidence of transient but asymptomatic bacteraemia in the first 24 hours after ingestion of the bacteria documented by detection of DNA in peripheral blood,[76] which may have represented the initial transit of bacteria from the gut mucosa to the lymphoid tissues prior to the incubation period. Associated with the initial DNAaemia is a systemic cytokine response, notably consisting of sCD40L, fractalkine (CX3CL1), GROα,

IL1RA, EGF, and VEGF occurred regardless of whether the individual later goes on to develop overt evidence of typhoid disease. This may represent inflammatory perturbation at the gut mucosa, perhaps implying that the infection is limited to the mucosa, but could also be consistent with invasive infection even among those who do not go on to show evidence of overt infection.[77] Onset of clinical invasive disease was heralded by a gradual fall in eosinophil count over the 5 days preceding onset of symptoms, followed by a fall in total white cell count, lymphocytes, neutrophils and platelets following the onset of clinical disease.[73] It is not clear whether these changes represent successful deployment of an appropriate immune and inflammatory response to the infection, or may be a failure of an appropriate protective response.

After the onset of febrile symptoms the profile of transcriptomic responses were driven by strong type I/II interferon signals that were associated with bacteraemia in the study.[77] There was evidence that this signalling interfered with tryptophan metabolism which may be part of the host response to limit bacterial growth. It was also shown that hepcidin levels increased and blood iron levels fell, as part of the acute innate response to infection, limiting iron availability for extracellular bacteria in the blood but increasing iron availability in macrophages supporting survival of internalised organisms, a key feature of the pathogenesis of typhoid fever.[78] Almost all individuals had positive blood cultures associated with diagnosis of infection in the model, with a median of 1 CFU/ml of blood detected.[73]

[H3] Antibody response

Among those challenged with *S.* Typhi who progressed to develop clinical disease, IgG, IgM and IgA responses to H (flagellar) antigen and lipopolysaccharide (LPS) were detected in peripheral blood, but there was no measurable anti-Vi antibody response in these naïve individuals.[73] Using a 250-antigen array, responses in the CHIM were further probed and identified that signatures containing flagellin, OmpA, HlyE, sipC, and LPS IgG IgM and IgA antibody responses could distinguish typhoid from other febrile illnesses in an endemic setting.[79] IgA to LPS antigen performed particularly well as a diagnostic marker in the model. The CHIM was also used to assess whether gene expression profiles might identify individuals with typhoid infection compared with controls, and a set of five gene profiles was identified that could distinguish typhoid from other febrile illnesses.[80]

[H3] Role of typhoid toxin.

Typhoid toxin may be an important virulence factor for *S.* Typhi, with studies suggesting that it may induce some of the hallmark clinical features of the disease in murine models.[81,82] It is, however, also found in other typhoidal and non-typhoidal Salmonellae including some that do not cause the clinical syndrome of enteric fever.[61,83,84] To assess its importance in virulence, volunteers were challenged either with a toxin-negative or wild-type strain.[85] There was no difference in proportions developing typhoid illness in the two groups but, unexpectedly, bacteraemia was more prolonged in the toxin-negative group. These observations suggest that typhoid toxin does not have an important role in susceptibility to typhoid infection.[85]

[H3] Infection-derived immunity

Immunity derived from infection with *S.* Typhi is likely to be an important factor in considering the impact of vaccination on transmission of the pathogen. Whilst modelling studies include this as an important variable there is little data on the level and duration of protection afforded by clinical disease episodes.[86,87] The CHIM was therefore used to investigate this question of infection-derived immunity. Volunteers who underwent a homologous rechallenge with *S.* Typhi or *S.* Paratyphi A, after prior CHIM infection (median 19 months previously, range 12-67 months), had a moderately reduced risk of developing typhoid (36%) or paratyphoid (57%), but there was no protection conferred by previous heterologous cross-challenge.[33] In those who did develop enteric fever, there was no difference between symptoms in naïve individuals (those not previously challenged) and those who had previously been challenged. Interestingly, baseline anti-LPS, anti-H and anti-Vi antibody levels were similar between the naïve and rechallenged groups, and there was no obvious boost in antibody in those with prior exposure.[33]

[H3] Stool Shedding

Stool shedding was studied across six typhoid and paratyphoid CHIM studies with 4,934 stool samples to identify factors which might reduce stool shedding and potentially reduce transmission in field settings.[88] Prior infection in those who were rechallenged in the CHIM was associated with reduced shedding (OR 0.30; 95% CI, 0.1–0.8) as was prior vaccination

with a Vi-containing vaccine (OR 0.34, 95% CI: 0.15–0.77 for Vi polysaccharide vaccine; and OR 0.41, 95% CI: 0.19–0.91 for TCV). There was also a non-significant reduction in shedding in the stool associated with the live oral Ty21a vaccine.

[H3] Accelerating Vaccine testing with CHIM

Along with improving our understanding of disease pathogenesis, the CHIM also provides a controlled method for testing novel vaccines at a lower cost and greater speed than large-scale traditional field trials. The Oxford CHIM has performed two such trials (Box1)

M01ZH09 vaccine

The CHIM model was used to study the efficacy of a novel oral live attenuated (deletion of ssaV and aroC genes) vaccine M01ZH09. The vaccine did not meet significance for protective efficacy; however, it did induce strong antibody responses against LPS, which were shown to be bactericidal. There was no association of antibodies with protection against infection, though they did associate with lower severity of symptoms, delayed onset of infection and a lower level of bacteraemia in vaccinees.[89] Similarly, vaccination of individuals prior to challenge with Vi-containing vaccines induced bactericidal antibodies, but these did not associate with protection from infection.[90] Duration of bacteraemia with the antibiotic-susceptible challenge strain was longer when treated with azithromycin compared with ciprofloxacin.[91]

Typhoid Conjugate Vaccine

A multi-arm phase 2b study comparing a novel TCV and a WHO pre-qualified and licensed Vi-polysaccharide (Vi-PS) vaccine against a control vaccine (that has no protective efficacy against *S.* Typhi) showed a comparable efficacy to the existing Vi-PS vaccine.[92] Extensive analysis of class, subclass, avidity and functional serological responses showed that Vi IgA levels and avidity associated with protection from *S.* Typhi challenge, and higher anti-Vi IgG responses were associated with reduced symptoms. In addition antibody-dependent neutrophil phagocytosis also associated with protection.[93,94] Vaccination with TCV induced alpha-4beta-7 and CCR10a positive IgA+ plasma cells indicating likely mucosal migration, which may be important since this is the site of invasion if there is a future exposure to the organism. Moreover, in those who received TCV, protection against infection was associated with the total plasma cell response.[95]

[H2] Antimicrobial Resistance

Antimicrobial resistance (AMR) is common in both *S.* Typhi and *S.* Paratyphi A, and is typically driven by local antibiotic prescribing.[96,97] Multidrug resistant (MDR) *S.* Typhi is defined as the combination of resistance to three previous first-line treatments; chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. MDR *S.* Typhi emerges through the simultaneous acquisition of multiple resistance genes encoded on a single transmissible plasmid, which can move between bacterial species and strains, and has been a clinical problem since the 1980s.[98] By the 1990s, in parts of south and southeast Asia the majority of *S.* Typhi infections were MDR,[99] prompting a switch to fluoroquinolones and azithromycin as the mainstays of treatment. However, fluoroquinolone resistance is now highly prevalent in these regions, mostly due to mutations in the *gyrA* and *parC* genes.[52,100] Extensively drug resistant (XDR) *S.* Typhi, defined as the combination of MDR plus resistance to fluoroquinolones and third-generation cephalosporins, has now emerged. A large outbreak of XDR *S.* Typhi was reported in Pakistan in 2016. The corresponding variant (4.3.1.1.P1) has now spread throughout the country and now caused the majority of typhoid cases reported there in 2018-2019.[101,102] This XDR variant has been detected in other countries, but is usually linked to recent travel to Pakistan[103,104] and at the time of writing does not appear to have become established elsewhere. MDR *S.* Typhi prevalence has declined below 10% in India and Nepal; however, as MDR plasmids still circulate amongst other *Salmonella* in these settings, a return to the older drugs is not favoured as it may prompt a return of MDR and subsequently XDR *S.* Typhi. Azithromycin resistance has been reported, mainly in south Asia, but remains rare (<1%).[57] In contrast, in sub-Saharan Africa, MDR *S.* Typhi is common in most countries, and fluoroquinolone resistance is increasing in countries where this drug class is used with a direct link to local fluoroquinolone prescribing patterns in Africa having been described;[105] azithromycin and XDR strains are, however, extremely rare.[47,53,96,97] *S.* Paratyphi A infections are rarely MDR, but are almost universally fluoroquinolone resistant.[44,46,96] Azithromycin resistance is reported in *S.* Paratyphi A in south Asia but remains rare, as it is in *S.* Typhi.

[H1] Diagnosis, screening, and prevention

[H2] Diagnosis

One major obstacle to controlling typhoid fever is the absence of reliable and easily deployable diagnostics. In most resource-constrained settings, it is diagnosed based on clinical symptoms and treated empirically and at the most the non-specific Widal test is used.[106] Most patients with typhoid fever present with nonspecific clinical features, with fever predominating, alongside symptoms such as malaise and headache.[107] Therefore, it can be challenging to differentiate typhoid fever from other febrile illnesses that impact typhoid endemic settings, such as malaria, dengue, or scrub typhus.[8] Multiple studies in typhoid-endemic areas in Asia have demonstrated that relying on clinical features to diagnose typhoid fever is unreliable with low specificity (< 15%) and positive predictive values (≤ 10%).[108,109]

Efforts are in progress to create a benchmark specification for an improved diagnostic test for typhoid fever. Ideally, this test would fulfill several key criteria: it would be inexpensive (for instance, costing less than $1), highly accurate (with a high sensitivity and specificity), quick (results available in less than 15 minutes), and user-friendly, requiring no data-interpretation, minimal training or sample processing, and not dependent on a stable water or power supply. Meeting these standards would significantly improve the clinical diagnosis and management of typhoid fever, thereby reducing its morbidity and mortality. It would also make a substantial contribution to combating antimicrobial resistance. The available tests for typhoid do not currently meet these specifications, but there are promising assays in development.

[H3] Culture diagnosis

Culture diagnosis from a normally sterile site (blood or bone marrow) is considered the reference standard for typhoid fever. However, it takes several days to get a result and requires substantial laboratory capacity not widely available in resource-constrained areas. The sensitivity of culture depends on the specimen type, prior antimicrobial use, timing of collection, and sample volume due to differences in bacterial burden at systemic sites.[110] The organism burden in bone marrow is orders of magnitude higher than in the peripheral blood (median of 10 vs. 0.5 colony forming units/mL, respectively),[111] and bacterial load in the blood peaks during the first week of infection.[110] Bone marrow culture has the highest

sensitivity (>90%)[112] and bacterial load remains high in bone marrow for several weeks, but this method has limited clinical utility due to its invasiveness. Blood culture has a sensitivity of only 50-70%,[110,113] and stool culture has a sensitivity of 30-40%.[114] In addition to having low sensitivity, a positive stool culture may indicate either acute disease, convalescent disease, or chronic carriage, and is therefore not considered diagnostic of current invasive disease.

[H3] Molecular testing

Molecular diagnostics have offered great promise to improve sensitivity and decrease time-to-result. Multiple nucleic acid detection methods have been developed including nested and multiplex conventional and real-time PCR and loop-mediated isothermal amplication; however, they suffer from many of the same limitations of blood culture.[115] Current PCR-based methods require laboratory capacity, and the stochasticity of genomes in small blood samples can lead to false negatives.[76,116] Due to the low magnitude of bacteremia common in typhoid fever, a pre-culture may be required to improve sensitivity.

[H3] Novel serodiagnostics

Commercially available serum-based diagnostics, including the Widal agglutination test and newer generation rapid diagnostic tests (RDTs), are widely available and detect antibodies against *S.* Typhi in serum or plasma. Although simple and fast, they have moderate sensitivity and specificity due to pre-existing antibodies from prior exposure and cross-reactivity. In a recent Cochrane Review of 37 typhoid RDTs, the best-performing assay, Tubex, had a sensitivity of 78% and specificity of 87%;[106] and a prospective and hybrid retrospective study of 9 commercially available RDTs, showed the best-performing test was Enterocheck with 73.8% sensitivity and 94.5% specificity.[117] These results underscore the need for improved tests that accurately detect the *S.* Typhi organism itself.

Advances in antigen discovery have, however, revealed several novel antigen targets to improve serodiagnostic assays.[79,118,119] Several of these antigens were further validated in populations from Bangladesh and Nepal[120] and led to the development of a promising RDT, the DPP® Typhoid Assay. This assay is based on detecting *S*. Typhi LPS and Hemolysin E-specific IgA, and early studies demonstrate sensitivity and specificity of >90%.[120] Other novel

typhoid diagnostic approaches currently being explored are host gene or metabolite signatures to discriminate typhoid from other febrile illnesses.[80,121]

[H2] Surveillance

Due to the lack of an optimal diagnostic test for typhoid fever, data on its burden are limited except from large burden studies performed recently.[21,27] Wastewater and sero-surveillance are powerful and low-cost tools that have been used to monitor community pathogen burden for several infections and are currently being evaluated for measuring *S.* Typhi exposure and transmission within populations. In addition, these approaches provide burden estimates that are not biased by care-seeking behaviours and measure both symptomatic and asymptomatic infections. Antibody levels to HlyE have been demonstrated to be an accurate serologic marker of recent typhoid infection.[120,122] A recent multisite study used population-based serologic data to this antigen coupled with a new statistical modelling approach to estimate enteric fever incidence.[122] These estimates correlated well with blood culture-based disease incidence but were generally more than 100-fold higher than the unadjusted blood-culture confirmed disease rates. An existing challenge for serosurveillance studies for typhoid fever is that the antigens presently used cannot differentiate *S.* Typhi from *S.* Paratyphi A. Vi can discriminate these *Salmonella* serovars, however its effectiveness is limited by low seroconversion rates following S. Typhi infection and the prevalence of Vi antibody within endemic communities. The introduction of Vi-based TCV will further complicate its use in seroepidemiology, as Vi antibodies cannot distinguish infection from vaccine-induced immunity.[122,123] Environmental surveillance, which uses culture or PCR-based methods to detect *S.* Typhi shed by infected individuals in sewage or present in water sources, does not have this limitation. However, the experience of environmental surveillance for *S.* Typhi has been mixed.[14] The organism burden of *S.* Typhi is much lower than for viral infections (e.g., SARS CoV-2), which is reflected in a less frequent detection of *S.* Typhi in wastewater samples.[14] Ongoing studies are being conducted to ascertain if there is a correlation between environmental detection of *S.* Typhi and clinical incidence. If confirmed, we will potentially have two cost-effective and scalable methods that could complement blood culture-based clinical surveillance and expand typhoid surveillance to areas without access to blood culture. A potential limitation to

consider for environmental surveillance, however, is that representative samples may be difficult to obtain in at-risk communities that lack sewage systems.

[H2] **Clinical Syndromes**

Typhoid fever is an outpatient disease in most areas of endemicity and generally presents as undifferentiated febrile illness.[3] Symptoms of typhoid fever manifest 10-14 days following infection, with generalized fever and malaise, abdominal pain with or without other symptoms including headache, myalgias, nausea, anorexia, constipation and less commonly, diarrhoea **(Figure 4)**.[107,124] The fever is classically described as 'step-wise', manifesting in the first week of illness.[125] On clinical examination, hepatosplenomegaly is common (29-50%) but not present in all cases, while diffuse abdominal tenderness and a coated tongue (a superficial white coating on the surface) is common (56-85%).[124] Additionally, rose-spots(a blanching erythematous rash containing culturable *S.* Typhi) are reported in the historical literature. The antibiotic era has changed some of the clinical features historically seen in typhoid fever, with significantly reduced prevalence of hepatosplenomegaly and rose spots[3,126].

[H3] Gastrointestinal complications

Severe complications, such as shock, jaundice, intestinal perforation (IP), intestinal haemorrhage and encephalopathy, can occur if antimicrobial treatment is delayed or inadequate.[31] IP is commonly reported as a sequalae of severe typhoid infection, with the primary site of perforation occurring in the terminal ileum, resulting from necrosis of infected Peyer's patches.[127,128] Increasing prevalence of IP have been documented in outbreak scenarios and in countries with increasing antimicrobial resistance.[129] Recognising this, the WHO have included guidance on the surveillance of IPs, recommending all instances be recorded in countries with endemic typhoid.[2] A recent systematic review for IP in Africa found the case fatality ratio (CFR) to be between 4.6% to 75% in included studies, the majority of studies however (79%) reported a fatality rate between 10% and 30%.[128] Treatment is surgical, and another review estimated the mean duration of hospitalisation secondary to IP to be 18.4 days.[130]

[H3] Neurological manifestations

Although rare, numerous neurological manifestations of enteric fever have been reported in the literature, including typhoid meningitis and encephalopathy.[131] A large outbreak of blood-culture confirmed typhoid fever was reported from the Malawi-Mozambique border in 2009 with an unusually high burden of neurological complications (13%) and high mortality rate (4%). Dysarthria, ataxia, upper motor neuron signs and altered mental status were identified in over 40 individuals.[132] Whilst it is rare to culture *S.* Typhi directly from the cerebrospinal fluid (CSF), it is thought the cortical irritation leading to clinical symptoms is mediated by the typhoid toxin.[133,134]

[H3] Other complications

Systematic reviews have highlighted other complications that occur in different age-groups of patients with typhoid fever. Hepatitis (36%), anaemia (71%) and leukocytosis (41%) are more common in children under 5 years of age, while altered mental status (30%), upper respiratory tract infection signs (22%) and abdominal pain or tenderness (70%) are more common in school-aged children.[107,134] Young children are more likely to present with diarrhoea than older children and adults, while constipation and intestinal perforations are more often seen in older age groups.[107,134] In addition, respiratory symptoms (cough or bronchopneumonia) or neurologic complications (e.g., encephalopathy, febrile seizures) are more commonly seen in children than adults. Within these reviews there is also geographical heterogeneity reported for common complications from typhoid fever, with anaemia more prevalent in South Asia and abdominal distension, ileus and perforation more prevalent in sub-Saharan Africa.[31,107]

The estimated pooled prevalence of all complications (defined as any unfavourable evolution of the disease) in hospitalised patients was 27% (95% CI, 21% - 32%)[135] with a mean overall case fatality of 4.45% (95% CI 2.85% to 6.88%).[136] The manifestation and severity of typhoid fever can differ depending on the patient's age and geographical region. Children bear the highest disease burden, with higher CFR and complications seen in Africa (mortality 5.4%) than in Asia (mortality 0.9%).[21,31] In Africa the mortality from IP was estimated to be 19.7% compared with only 4.6% in Asia.[31] This observation on differential mortality rates between Africa and Asia is likely to be multi-factorial. Delays in accessing

healthcare, receiving an accurate diagnosis and administering appropriate treatment due to poorer healthcare infrastructure will almost certainly be contributory.[31]

[H2] Chronic Carriage

Historical data provide estimates that 2-5% of acutely infected individuals develop typhoid chronic carriage, although this was in the era prior to fluoroquinolone antimicrobials, and more recent estimates may be less.[137,138]

To establish long-term carriage, organisms must enter the biliary tract either directly by ascending through a malfunctioning sphincter of Oddi, or via the liver during systemic infection.[139] Epidemiological investigations through case-control studies, and ultrasound imaging in both mice and human participants, have shown the association between the carrier state and the development of bacterial biofilm on gallstones within the gallbladder by *S.* Typhi bacteria.[140-142] This is supported by data from diverse global contexts showing prevalence of chronic carriers increase with age, and are predominantly female, risk factors which mirror the development of gallbladder pathology.[37,137,141]

The importance of carriage in low incidence, non-endemic settings has been shown through multiple outbreaks which have been traced to a chronic carrier often responsible for food preparation.[143] However, the contribution of carriers to ongoing transmission within endemic sites and the diagnosis of these individuals remains unclear. Shedding of the pathogen within stool is intermittent, and at a low level, meaning detection through serial stool culture is both programmatically difficult and has a low sensitivity.[144]

Isolating the organism directly from the gallbladder is the gold standard for diagnosing carriage. This may be useful in individuals undergoing cholecystectomy but is highly impractical at a public health level due to the invasive nature of the procedure. The duodenal string test, where a capsule is passed into the stomach, allowing a nylon string to pass through the pylorus and into the duodenum allowing the collection and subsequent culture of duodenal and bile fluid, has been used historically for both the diagnosis of acute and chronic typhoid.[145,146] However, once again, this is impractical at a public health level due to the invasiveness and inconvenience of the test.[37]

The use of serological screening for chronic carriage, using anti-Vi antibody, has been successful in non-endemic sites[143,147] but in areas of medium to high incidence, where

regular infection or exposure to the pathogen increases the Vi titre, this has had mixed results.[148-150] More recent work has been done to identify novel serological markers of acute typhoid and carriage, along with transcriptomic and a metabolomic profile, which could improve the prospective diagnosis of this population.[151-153]

[H2] Prevention

[H3] Vaccine Development

Typhoid vaccines have been in use since the late 19th century with heat-killed whole cell vaccines developed.[154] Unfortunately, the systemic and local side effects from these earliest vaccines rendered them unusable in young children, resulting in significant school absenteeism.[155] Two further vaccines were developed in the second half of the 20th century. The oral live-attenuated Ty21a vaccine has a pooled efficacy of 50% after 3 years of follow-up in meta-analysis.[156] multiple doses are required, and the capsule formation makes it difficult to administer to children 6 years of age and younger. The Vi-polysaccharide (Vi-PS) is a parenteral vaccine containing the purified capsular Vi-polysaccharide antigen. Combining results from individually and cluster randomised trials, efficacy at 2 years for Vi-PS was 59%.[157] Vi-PS is not licensed in children <2 years of age due to poor immunogenicity. Since the vaccine contains a T-cell-independent antigen, it also does not induce strong cellular immunological memory, and cannot be boosted with repeated vaccination. Although these vaccines have been widely used for travellers, these limitations mean that they have not been widely adopted outside of outbreak control in low-income settings despite a WHO recommendation in 2008 for their use to improve typhoid control.[155]

[H3] Typhoid Conjugate Vaccines

More recently, a new generation of typhoid conjugate vaccines have become available, in which the polysaccharide Vi capsule is chemically conjugated to a protein carrier, therefore producing a T-cell-dependent response with a greater and longer-lasting immunogenicity, including among younger children and infants from 6 months of age. Such conjugate vaccines have demonstrated high levels of protection for other pathogens, including *Haemophilus influenzae* type b (Hib), pneumococcus and meningococcus.[158] In 2018 the

WHO therefore published a fresh recommendation for use of new-generation TCV in countries with either a high burden of disease, or high prevalence of antimicrobial resistance, or both.[159] Notably, this was the first vaccine with a WHO recommendation explicitly based on the potential to prevent the spread of antimicrobial resistance. The recommendation is for a single dose of TCV for children from 6 months of age, introduced into country routine immunization schedules alongside mass catch-up campaigns from the first or second year of life through to 15 years of age.[159]

[H3] Randomised Clinical Trials

Licensure of the first TCV was based on an immunogenicity and safety trial from India,[160] with the first vaccine efficacy data coming in adults in a non-endemic setting, as part of the Oxford CHIM for typhoid. With a composite endpoint of prolonged fever followed by blood-culture confirmation of *S.* Typhi, reflecting clinical settings, this trial showed an efficacy of 87.1% for TCV, compared with 52.3% for the comparator, Vi-PS.[92,161]

Since this point, data from several phase 2 and 3 clinical trials in diverse high-burden endemic settings confirm excellent safety, immunogenicity (including safe co-administration with other routine immunisations) and efficacy for TCV among children (Table 2).[162,163] In Malawi, in an individually randomised controlled trial (RCT)[164] in children 9 months through 12 years of age, TCV was found to be 81% effective in intention to treat analysis, and 84% effective in per protocol after 18 to 24 months of follow-up.[165] Longer-term efficacy data after more than 4 years of follow-up have shown an overall intention-to-treat efficacy of 78% from the same cohort, suggesting durable protection (Lancet pre-print https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4411421). These data showed significant protection from vaccination for children under 2 years of age, important for a vaccine that will also be used in routine immunisation in the first 2 years of life. In a similar trial from Nepal,[166] in children 9 months through 16 years of age, a similar efficacy of 79% was seen after 2 years of follow-up.[167] Typhoid vaccines might have the potential to prevent not only disease, but also transmission, and cluster randomised trials are designed to evaluate both individual protection of vaccinated individuals, and also indirect “herd” protection (in non-vaccinated individuals living in the same area). In a cluster randomised trial from Bangladesh[168] in children 9 months through 16 years, individual protection from TCV was 85%, with overall protection (protection of all residents within vaccine clusters) of

57%, and indirect “herd” protection (of non-vaccinated participants within vaccine clusters) of 19%, which did not reach statistical significance.[169] To evaluate the possibility that the lack of significance could have been caused by migration of cases between clusters, a further analysis of this trial was conducted using the ‘fried egg’ approach, in which the whole cluster receives the allocated treatment but only the inner area of the cluster (the “egg yolk”) is used for analysis, as this inner area should be less affected by spillover from neighbouring clusters with different vaccine allocation. This re-analysis, again, did not suggest any significant additional indirect protection among non-vaccinated individuals, and the authors concluded that in the absence of indirect protection, vaccination campaigns across a wider age-range, to include adults, might be required in some epidemiological settings to achieve typhoid control.[170] Nevertheless, the individual protection afforded by TCVs between these 3 large vaccine efficacy trials, in comparable age-groups, and across three very epidemiologically diverse sites is strikingly consistent.

Alongside these clinical trials, data have been published from post-vaccine introduction evaluations, from countries such as India,[171] Pakistan[172] and Zimbabwe.[173] Data from Pakistan provide confidence that TCV is highly effective against the XDR strain of *S.* Typhi, providing evidence that as well as reducing the burden of typhoid fever, it will have a positive impact on decreasing antimicrobial resistance.[174]

While the safety, immunogenicity and efficacy of TCVs has been demonstrated in diverse populations, TCVs alone are unlikely to eliminate typhoid fever, as evidenced by the incidence rates in the vaccine groups of the trial populations. Thus, their use should be viewed as an important adjunct to improvements in WASH, as the latter has successfully eliminated typhoid fever in many countries around the world.[175-177]

[H1] Management

Antimicrobials transform this prolonged febrile illness, that can have a mortality between 10 and 30%, to an illness where symptoms resolve within a week with a case fatality ratio <1%.[126] The emergence of resistance to all the commonly used antimicrobials for treating enteric fever challenges this picture.[178] AMR is associated with treatment failure, an increased risk of complications, and increased potential for transmission due to prolonged

744 fecal carriage.[126,179,180] Treatment choices should take account of local antimicrobial
745 resistance patterns, if known, and national guidelines where available.[181]
746
747 **[H2] Antimicrobial therapy**
748 Most patients with enteric fever are treated with an oral antimicrobial as part of outpatient
749 management in the first week of illness and then recover within a week. The WHO Essential
750 Medicines Expert Committee concluded that a seven-to-ten-day course of either
751 ciprofloxacin, ceftriaxone or azithromycin should be considered first-choice treatments on
752 the core list of the Essential Medicines List for adults (EML) and EML for children.[182]
753 Ciprofloxacin is not a suitable choice in much of south Asia, and some areas of sub-Saharan
754 Africa, because of widespread resistance (indicated by resistance to nalidixic acid, pefloxacin
755 or ciprofloxacin).[126,178] Azithromycin is an effective alternative drug although there are
756 sporadic reports of resistance.[183,184] For children, patients admitted to hospital, and when
757 resistance to other drugs is uncertain, parenteral ceftriaxone has been a safe option.
758 Although oral chloramphenicol, amoxicillin and trimethoprim-sulphamethoxazole were
759 commonly used prior to the 1990s, MDR, with plasmid mediated resistance to all these
760 three options, appeared in the late 1980s and became widespread.[97]
761
762 Systematic reviews of the comparative efficacy of chloramphenicol, the fluoroquinolones
763 (such as ciprofloxacin, ofloxacin, and gatifloxacin), azithromycin, and cephalosporins (such
764 as ceftriaxone and cefixime) in typhoid fever treatment have been unable to draw firm
765 conclusions on the presence or absence of important differences between the different
766 antimicrobials.[185-187] Much of the randomised controlled trial evidence is of low certainty
767 because individual randomised controlled trials have been small, with methodological
768 problems such as not double-blinded, and conducted >20 years ago. The lack of diagnostic
769 sensitivity of blood culture, the paucity of trials in the outpatient setting, the changing
770 pattern of resistance over time, and the lack of agreed core outcome indicators are further
771 limitations.
772
773 **[H2] Antimicrobial resistant strains**
774 Confidence in ceftriaxone has been impacted by an outbreak of XDR *S.* Typhi in Pakistan
775 since 2016.[188] These strains are resistant to chloramphenicol, ampicillin/amoxicillin

trimethoprim-sulphamethoxazole, ciprofloxacin and ceftriaxone/cefixime but susceptible to oral azithromycin and parenteral meropenem.[101] The cephalosporin resistance is mediated by carriage of a plasmid mediated *bla*$_{CTX\text{-}M\text{-}15}$ extended spectrum beta-lactamase (ESBL) gene.[189] These infections have also been seen in other countries in travellers from Pakistan[103] and sporadic reports of ceftriaxone resistance have been seen in other locations.[190,191] Clinicians treating patients with XDR *S.* Typhi have found no important differences in the clinical response between oral azithromycin alone, intravenous meropenem alone and a combination of azithromycin and meropenem.[192] Notably, the daily cost of meropenem in Pakistan was 15 times more than azithromycin.

[H2] Combination therapy

Studies of the number and location of bacteria in typhoid fever have confirmed both an intracellular and extracellular location of the bacteria with high numbers in sites of the reticuloendothelial system such as the bone marrow.[111,193] Antimicrobials used to treat typhoid fever should target all these locations. Combining azithromycin, which reaches very high intracellular concentrations but low extracellular concentrations[194] with a beta-lactam antimicrobial predominantly active in the extracellular compartment has been suggested as a better option for the treatment of typhoid fever. In an RCT of 105 adults with confirmed typhoid fever in Nepal, a combination of azithromycin and cefixime for outpatients and azithromycin and ceftriaxone for inpatients was superior to azithromycin alone with shorter fever clearance times.[195] An ongoing clinical trial is examining the efficacy of an azithromycin and cefixime combination in suspected cases of enteric fever in south Asia.[196]

[H2] Severe infections

In severe typhoid fever, supportive care is critical to the outcome. This may include full intensive care support, blood transfusion in the event of gastrointestinal haemorrhage and surgery if there is an intestinal perforation and peritonitis.[197] Following perforation, there may be secondary blood stream infection with a range of pathogens from the gut lumen, requiring repeat of blood culture and broadening of antimicrobial cover. One RCT in Indonesia suggested high-dose methyl-prednisolone reduced mortality in severe typhoid characterised by altered consciousness and haemodynamic shock.[198] Methodological issues

make it difficult to draw definitive conclusions from this study and further trials on this question are needed.[199]

[H2] Chronic carriers

Chronic faecal carriers can shed large numbers of bacteria in the faeces and be a source of infection to others in the community.[200] A systematic review of studies of the antimicrobial treatment of chronic carriage identified eight studies but only one RCT.[201] Fluoroquinolones are effective in eradicating chronic carriage of susceptible isolates after a 28-day course. The only double-blinded RCT performed showed an eradication rate of 92% in those given a 28-day course of norfloxacin compared to 11% in those given placebo. Six studies evaluated ampicillin or amoxicillin in a four-to-six-week course with cure rates around 70%. Cholecystectomy may be an option where eradication has failed, particularly in the presence of structural biliary abnormalities including gallstones which may provide a protected niche for bacteria, but should be balanced with the risk of surgical complications.[126] All these studies pre-dated the emergence of widespread MDR and FQ resistance, and further clinical trials in this area, for example using azithromycin, would help guide modern management.

[H1] Quality of life

[H2] Cost of Illness

Despite the potential acute effects and sequelae from typhoid fever, the disease's toll on quality of life is not well documented. However, a number of studies have assessed the economic burden of typhoid in terms of costs to healthcare providers and to affected households in low- and middle-income countries.[202-206] A review of economic evidence highlights the cost of hospitalisation as the most common expense reported in the literature. Costs per hospitalised case range from $159 to $636 in India, $233 in Nepal, and $171 in Tanzania (2016 US$).[207-210] Costs for treating outpatients ranged from $0 to $14.1 (2010 US$). [211]

The cost of intestinal perforations, a complication that may result from untreated typhoid or delayed access to care, has been specifically studied in a few countries. The additional

surgical costs required to repair an intestinal perforation, on average, are as high as $452 in Nigeria and $1,210 in India (2019 US$). Those high costs are accompanied by longer hospital stays, 23 days on average in Nigeria and 19 days in India, which also increase a family's expenses.[212,213]

The potential for higher cost of illness associated with MDR and XDR *S.* Typhi infection, requiring more expensive and less available treatments, is not well documented. Data from the XDR outbreak in Pakistan between 2016 and 2018, however, suggest that the cost of an episode of typhoid from XDR *S.* Typhi is 2 to 4 times higher than the cost of non-XDR *S.* Typhi infection.[214]

Due to the difficulty in diagnosing typhoid, health care seeking can be a long and costly endeavour for patients and their caregivers. Households often face indirect expenditures such as transportation, loss of household income, and food and subsistence costs related to seeking and receiving care, alongside direct out-of-pocket costs including diagnosis and treatments. Typhoid predominantly impacts children younger than 15 years old, which means a case of typhoid often results in parental absenteeism from work, and a loss of income for caregivers, which can cause financial consequences for families. These expenses may reduce expenditures on other household spendings, which can affect investments in nutrition, education, and other household needs, and trigger dis-saving measures, resulting in long-term adverse socioeconomic impact.

Typhoid can represent a catastrophic cost to affected families, defined as expenses and loss of revenue due to seeking care or caring for sick children and family members that represents more than 40% of non-food monthly household expenditure. A recent study in Malawi reported that, despite free access to all government medical care and minimal out-of-pocket direct healthcare costs, 44% of households faced catastrophic illness costs mainly related to indirect costs, and 16% of households experienced illness costs that were more than their total monthly income.[215] The median cost per case for enteric fever patients reporting inpatient care costs was also determined as catastrophic for families in studies in Bangladesh, Nepal, and Pakistan.[207-210] As well as revealing the sadder human and societal

costs of typhoid, cost of illness estimates are essential for evaluation of vaccine cost effectiveness, to inform policy decisions (Box 1).

7. Outlook

Considerable progress has been made over the past decade in the development and licensure of TCVs supported by robust evidence on safety and immunogenicity, innovative data on efficacy from the typhoid human challenge model, and field efficacy data from large clinical trials conducted in diverse populations at risk.[216] The gather of a compelling body of data prompted and has reaffirmed WHO recommendations on use of single dose TCVs in endemic settings.[217,218] TCV is well-tolerated and may be co-administered with other childhood vaccines, facilitating its integration into the WHOs Expanded Programme on Immunisation (EPI) at 9-18 months of age. For low resource countries, Gavi (the vaccine alliance) will co-finance introduction of TCV into EPI, and fully finance single dose catch-up campaigns for all children to 15 years of age.[219] Country introductions have begun in both Africa and Asia, however, most at-risk children globally remain without protection. To this end, a coordinated multidisciplinary approach that includes advocacy and communications; country support for decision-making, preparation of Gavi applications and planning of vaccine delivery; and an adequate stable manufactured supply of prequalified vaccine is essential to ensure that more children are protected from this disease sooner.

Several recent studies have assessed the cost-effectiveness of TCV introduction in typhoid-endemic countries. In endemic areas, incorporating TCV into the routine immunisation schedule at 9 months of age with an initial catch-up campaign to 15 years of age has generally been found to be cost-effective from the healthcare provider perspective at willingness-to-pay thresholds comparable to other recently introduced vaccines in high-incidence settings.[211,220,221] When factoring in the indirect costs to patients, TCVs may even be cost-saving from the societal perspective, as has been found for urban areas in India.[222,223] However, TCV introduction is unlikely to be cost-effective in settings where the incidence of typhoid fever is low, at <50 cases per 100,000 person-years.[211,220,222]

Two TCV products are prequalified by WHO and are considered equally effective. Several more are approved nationally or are under development. However, as with pneumococcal conjugate vaccines, robust data on relative effectiveness of different products is important to provide confidence to policymakers on use of different vaccines, highlighting the importance of ongoing impact studies in settings where TCV has been introduced. These studies will inform the long-term TCV strategy.

Perhaps the most important outstanding scientific question about the global TCV programme is the duration of protection. While the TCV efficacy trials have shown robust and durable protection against disease, of around 80%, for more than 4 years after vaccination in clinical trials covering pre-school and school-age children (Lancet pre-print https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4411421), longer term protection studies are needed for those younger children immunised with a single dose of vaccine at 9-18 months in the EPI schedule. Ongoing longer-term post-introduction effectiveness and impact studies may strengthen evidence in this domain. Given the high rates of disease reported among school-age children, the need for a booster prior to school entry, among those vaccinated in early-life routine immunisation programmes, must be assessed in Africa and Asia in countries where the vaccine is being rolled out.

It remains unknown who in the population is primarily responsible for transmission. The lack of evidence of significant indirect protection in a cluster-randomised trial in Bangladesh in which children under the age of 16 were vaccinated implies that either the vaccine prevents clinical illness but does not prevent transmission, or that adults also contribute substantially to transmission.[149,150] Alternatively, the complexities and biases in a cluster randomised design in an urban setting may make it impossible to detect herd effects that are present. Such information could help inform whether extending vaccination to older age groups may provide additional population-level benefits. It also remains possible that targeted vaccination of those adults responsible for transmission could improve typhoid control in high burden settings. Ongoing observational studies in countries implementing TCVs may provide further evidence to address this question in the next 5 years.

Improved diagnostics are needed both for clinical management of disease, and to define burden and inform decision-making on TCV introduction. Innovation and flexibility is needed

to ensure that the most disadvantaged children, who may not have access to blood culture facilities, do have access to TCV. Further, without diagnostics, the impact of TCV may be less apparent in South Asia, for example, where rates of paratyphoid infection can be substantial and symptoms are indistinguishable from typhoid. Work on paratyphoid vaccines, combined with TCV, could broaden protection if shown to be effective and reduce the overall enteric fever burden further. With ongoing early safety and immunogenicity studies of bivalent typhoid/paratyphoid vaccines already underway, a combined vaccine could be available within the next 5 years. Combination of TCVs with vaccines targeting other bacterial enteric pathogens could broaden the impact of vaccine programmes, and there are now early phase studies with multivalent vaccines combining TCV with emerging vaccines against iNTS disease.

Despite the huge progress in protecting children against typhoid with TCVs, the ongoing transmission of these *Salmonella* and other bacterial pathogens in affected populations can only be fully controlled with improvements in water, sanitation, and hygiene (WASH) and food safety. Improving and maintaining WASH requires considerable financing, structural change, and political commitment, and some of the least-developed countries have experienced entrenched poor sanitation for decades. The impacts of climate change may not only alter the environmental and household patterns of transmission of typhoid, but will also likely further heighten the challenge of delivering sustained improvements in WASH.

The global rise of antimicrobial resistance further adds relevance and urgency to the role of vaccines, and the sparsity of new antimicrobial products in development also underscores the need for all available means of control to be mobilised. The remarkable recent efforts in typhoid immunisation programmes will help protect at-risk children in the face of these global challenges.

Table 1: Case Definitions for typhoid fever disease states, adapted from World Health Organisation Typhoid Vaccine Preventable Disease Surveillance Standards, 2018. https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-typhoid

Confirmed Case Acute Typhoid Fever	Laboratory confirmation by culture or molecular methods of *S*. Typhi or detection of S. Typhi DNA from a normally sterile site
Relapse of Typhoid Fever	Laboratory confirmation of *S*. Typhi from a normally sterile site within one month of completing an appropriate course of antimicrobial treatment and resolution of symptoms.
Chronic Typhoid Carrier	Evidence of shedding of *S*. Typhi (positive stool culture or PCR) at least 12 months after finishing an appropriate course of antimicrobial treatment and the resolution of symptoms following a laboratory-confirmed episode of acute disease OR Two stool samples 12 months apart positive for *S.* Typhi.
Convalescent Carrier	Evidence of shedding *S*. Typhi (positive stool culture or PCR) 1–12 months after finishing an appropriate course of antimicrobial treatment and the resolution of symptoms following a laboratory-confirmed episode of acute disease
Suspected Case of Typhoid	Fever for at least three out of seven consecutive days in an endemic area or following travel from an endemic area OR Fever for at least three out of seven consecutive days within 28 days of being in household contact with a confirmed case of typhoid or fever

Table 2: Summary table summarising findings from published efficacy and effectiveness estimates for TCV.

Country	Design	Control vaccine	Age	Study period	Duration of follow-up	Number enrolled	Efficacy (95% CI)
Malawi efficacy	Individually-randomized	MCV-A	9 months-12 years	Feb 2018-Apr 2020	18-24 months	28,130	80.7% (64.2% to 89.6%)[165]
				Feb 2018-Sept 2022	4.3 years		78.0% (66.3% to 86.1%)
Nepal efficacy	Individually-randomized	MCV-A	9 months-15 years	Nov 2017-Apr 2018	6 months	20,019	81.6% (58.8% to 91.8%)[167]
				Nov 2017-Feb 2020	24 months		79.0% (61.9% to 88.5%)[224]
Bangladesh efficacy	Cluster randomized	JE (SA 14-14-2)	9 months-16 years	Apr 2018-May 2020	17.1 months	~ 67,500	Total protection 81% (39.0% to 94.0%) Overall protection 56% (43.0% to 68.0%) Indirect protection 19% (-12% to 41%)[169]
India Effectiveness	Cluster randomized Test Negative	NA	9 months-14 years	Sept 2018-Mar 2021	31 months	NA	Programmatic overall effectiveness 56% (25% to 74%)[171]
Pakistan Effectiveness	Cohort	NA	6 months-10 years	Feb 2018-Dec 2019	23 months	NA	Culture confirmed *S.* Typhi 95.0% (93.0% to 96.0%) XDR S. Typhi 97.0% (95.0% to 98.0%)[225]
Zimbabwe Effectiveness	Case control	NA	6months-15 years	July 2019-March 2020	9 months	NA	84% (57.0% to 94.0%[173]

Box 1: Patient experience

Bashir's experience with typhoid

I am a 10-year-old boy from Badin, Sindh province, Pakistan. My ten siblings and I have never been to school. My father is a vegetable seller and earns about three to four dollars a day – which is only enough for two meals – so we stay at home, helping him with his work or playing with friends.

One day, while playing cricket, I found I had little energy to run. I returned home and told my mother that I was feeling unwell. I rested in bed for days, but my temperature kept increasing. My father took me to a nearby doctor who gave me medication and charged us six dollars. Even with the medication my body was still burning like an oven. I went to another doctor, who gave me a blood test and diagnosed me with typhoid. He charged us 27 dollars and prescribed more medication. After taking it, my condition continued to worsen. I began vomiting, feeling pain in my stomach, and was unable to even take a sip of water.

I was taken to a hospital in Badin, despite my family not having money for transportation or hospital care. There, I was told my intestine had burst and only a major surgery could save my life. We did not have the money for this procedure. I cried while thinking my life was about to end. An ambulance driver, who I think may be a guardian angel, suggested we travel to the National Institute of Child Health in Karachi, where patients are treated at almost no cost.

Accompanied by my family, we reached Karachi via ambulance and paid $45 for the four-hour journey. I underwent surgery the same night and began my recovery. I feel like I have been given another chance at life.

Figure legends

Figure 1. Incidence rates per 100,000 person-years of observation for typhoid fever, by country, in 2019.
This figure demonstrates the incidence of typhoid fever across the world, with the highest incidence areas shown in red, and low incidence areas in blue.

Map generated from Global Health Data Exchange VizHub GBD 2019: https://vizhub.healthdata.org/gbd-compare/.(Created by select map, 'use advanced settings;' display: cause A.3.2.1 Typhoid fever; measure: incidence; year: 2019; age: all ages; sex: both; units: rate).

Figure 2: Salmonella Typhi genotype prevalence by world region, 2010-2020.
This figure demonstrates the prevalence of genotypes of S. Typhi across the world. Countries contributing data are shaded in beige, and are grouped by regions as defined by the UN statistics division. These data are based on assumed acute cases isolated from untargeted sampling frames from 2010 onwards, with known country of origin (total N=9,478 genomes).
Carey, M. E. *et al.* Global diversity and antimicrobial resistance of typhoid fever pathogens: insights from 13,000 Salmonella Typhi genomes. *medRxiv*, 2022.2012.2028.22283969

Figure 3: Pathogenesis of Typhoid Fever following pathogen ingestion
A schematic figure relating the clinical presentation of typhoid fever to the pathogenesis describing the mechanisms of invasion and systemic dissemination.

Figure 4: Clinical signs and symptoms of typhoid fever.
Typhoid fever presents predominantly with fever, headache and abdominal pain, but symptoms and signs can be heterogenous and can include all organ systems

REFERENCES

1 Dougan, G. & Baker, S. Salmonella enterica Serovar Typhi and the Pathogenesis of Typhoid Fever. *Annual Review of Microbiology* **68**, 317-336, doi:10.1146/annurev-micro-091313-103739 (2014).

2 Organisation, W. H. *Typhoid: Vaccine Preventable Diseases Surveillance Standards*, <https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-typhoid> (2018).

3 Basnyat, B., Qamar, F. N., Rupali, P., Ahmed, T. & Parry, C. M. Enteric fever. *Bmj* **372**, n437, doi:10.1136/bmj.n437 (2021).

4 Von Drigalski, K. W. *Ueber Ergebnisse bei der Bekämpfung des Typhus nach Robert Koch*. (1904).

5 Kirchhelle, C., Pollard, A. J. & Vanderslott, S. Typhoid-From Past to Future. *Clin Infect Dis* **69**, S375-s376, doi:10.1093/cid/ciz551 (2019).

6 Ames, W. R. & Robins, M. Age and Sex as Factors in the Development of the Typhoid Carrier State, and a Method for Estimating Carrier Prevalence. *Am J Public Health Nations Health* **33**, 221-230, doi:10.2105/ajph.33.3.221 (1943).

7 Stanaway, J. D. *et al.* The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet Infectious Diseases* **19**, doi:10.1016/S1473-3099(18)30685-6 (2019).

8 Crump, J. A. Progress in Typhoid Fever Epidemiology. *Clin Infect Dis* **68**, S4-s9, doi:10.1093/cid/ciy846 (2019).

9 Appiah, G. D. *et al.* Typhoid Outbreaks, 1989-2018: Implications for Prevention and Control. *Am J Trop Med Hyg* **102**, 1296-1305, doi:10.4269/ajtmh.19-0624 (2020).

10 Reller, M. E. *et al.* in *39th Annual Meeting of the Infectious Diseases Society of America.* (ed IDSA) 199 (Infectious Diseases Society of America, Alexandria, VA).

11 Mitscherlich, E. & Marth, E. H. in *Microbial survival in the environment* (eds E. Mitscherlich & E.H. Marth) 1-560 (Springer, 1984).

12 Cho, J.-C. & Kim, S. J. Viable, but non-culturable, state of a green fluorescence protein-tagged environmental isolate of *Salmonella* Typhi in groundwater and pond water. *FEMS Microbiol Lett* **170**, 257-264 (1999).

13 Kingsley, R. A. *et al.* Functional analysis of *Salmonella* Typhi adaptation to survival in water. *Environ Microbiol* **20**, 4079-4090 (2018).

14 Andrews, J. R. *et al.* Environmental surveillance as a tool for identifying high-risk settings for typhoid transmission. *Clin Infect Dis* **71 (suppl 2)**, S71-S78 (2020).

15 Deen, J. *et al.* Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. *Lancet Infect Dis* **12**, 480-487 (2012).

16 Reddy, E. A., Shaw, A. V. & Crump, J. A. Community acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* **10**, 417-432 (2010).

17 Marchello, C. S., Dale, A. P., Pisharody, S., Rubach, M. P. & Crump, J. A. Prevalence of community-onset bloodstream infections among hospitalized patients in Africa and Asia: a systematic review and meta-analysis. *Antimicrobial agents and chemotherapy* **64**, e01974-01919 (2019).

18 Garrett, D. O. *et al.* Incidence of typhoid and paratyphoid fever in Bangladesh, Nepal, and Pakistan: results of the Surveillance for Enteric Fever in Asia Project. *Lancet Glob Health* **10**, e978-988 (2022).
19 Marks, F. *et al.* The Severe Typhoid in Africa Program: incidences of typhoid fever in Burkina Faso, Democratic Republic of Congo, Ethiopia, Ghana, Madagascar, and Nigeria. Available at SSRN: https://ssrn.com/abstract=4292849 or http://dx.doi.org/4292810.4292139/ssrn.4292849 (2022).
20 Marks, F. *et al.* Incidence of invasive *Salmonella* disease in sub-Saharan Africa: a multicentre population-based surveillance study. *Lancet Glob Health* **5**, e310-323 (2017).
21 Meiring JE, e. a. Burden of enteric fever at three urban sites in Africa and Asia: a multicentre population-based study with 626,219 person-years of observation. *Lancet Glob Hlth* (2021).
22 John, J. *et al.* Burden of Typhoid and Paratyphoid Fever in India. *New England Journal of Medicine* **388**, 1491-1500, doi:10.1056/NEJMoa2209449 (2023).
23 Crump, J. A., Luby, S. P. & Mintz, E. D. The global burden of typhoid fever. *Bull World Health Organ* **82**, 346-353 (2004).
24 Antillón, M. *et al.* The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. *PLoS Negl Trop Dis* **11**, e0005376 (2017).
25 Mogasale, V. *et al.* Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. *Lancet Glob Health* **2**, e570-580 (2014).
26 Buckle, G. C., Walker, C. L. & Black, R. E. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. *J Glob Health* **2**, 10401 (2012).
27 GBD 2017 Typhoid and Paratyphoid Collaborators. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Infect Dis* **19**, 369-381 (2019).
28 Crump, J. A. & Kirk, M. D. Estimating the burden of febrile illnesses. *PLoS Negl Trop Dis* **9**, e0004040 (2015).
29 Marchello, C. S., Hong, C. Y. & Crump, J. A. Global typhoid fever incidence: a systematic review and meta-analysis. *Clin Infect Dis* **68 (suppl 2)**, S105-S116 (2019).
30 Crump, J. A. *et al.* Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg Infect Dis* **9**, 539-544, doi:10.3201/eid0905.020428 (2003).
31 Marchello, C. S., Birkhold, M. & Crump, J. A. Complications and mortality of typhoid fever: A global systematic review and meta-analysis. *J Infect* **81**, 902-910, doi:10.1016/j.jinf.2020.10.030 (2020).
32 Marchello, C. S., Birkhold, M. & Crump, J. A. Complications and mortality of typhoid fever: A global systematic review and meta-analysis. *J Infect* **81**, 902-910 (2020).
33 Gibani, M. M. *et al.* Homologous and heterologous re-challenge with Salmonella Typhi and Salmonella Paratyphi A in a randomised controlled human infection model. *PLoS Negl Trop Dis* **14**, e0008783, doi:10.1371/journal.pntd.0008783 (2020).

34 Im, J. *et al.* Protection conferred by typhoid fever against recurrent typhoid fever in urban Kolkata. *PLoS Negl Trop Dis* **14**, e0008530, doi:10.1371/journal.pntd.0008530 (2020).

35 Brockett, S. *et al.* Associations among water, sanitation, and hygiene, and food exposures and typhoid fever in case-control studies: a systematic review and meta-analysis. *Am J Trop Med Hyg* **103**, 1020-1031 (2020).

36 Tadesse, B. T. *et al.* Prevention of typhoid by Vi conjugate vaccine and achievable improvements in household water, sanitation, and hygiene: evidence from a cluster-randomized trial in Dhaka, Bangladesh. *Clin Infect Dis* **75**, 1681-1687 (2022).

37 Gunn, J. S. *et al.* Salmonella chronic carriage: epidemiology, diagnosis, and gallbladder persistence. *Trends Microbiol* **22**, 648-655, doi:10.1016/j.tim.2014.06.007 (2014).

38 Dunstan, S. J. *et al.* Variation at HLA-DRB1 is associated with resistance to enteric fever. *Nat Genet* **46**, 1333-1336 (2014).

39 Saad, N. J. *et al.* Seasonal dynamics of typhoid and paratyphoid fever. *Sci Rep* **8**, 6870 (2018).

40 Thindwa, D., Chipeta, M. G., Henrion, M. Y. R. & Gordon, M. A. Distinct climate influences on the risk of typhoid compared to invasive non-typhoid Salmonella disease in Blantyre, Malawi. *Sci Rep* **9**, 20310, doi:10.1038/s41598-019-56688-1 (2019).

41 Levy, K., Smith, S. M. & Carlton, E. J. Climate Change Impacts on Waterborne Diseases: Moving Toward Designing Interventions. *Curr Environ Health Rep* **5**, 272-282, doi:10.1007/s40572-018-0199-7 (2018).

42 Gao, Q. *et al.* Impact of Temperature and Rainfall on Typhoid/Paratyphoid Fever in Taizhou, China: Effect Estimation and Vulnerable Group Identification. *Am J Trop Med Hyg* **106**, 532-542, doi:10.4269/ajtmh.20-1457 (2021).

43 Hickman-Brenner, F. W. & Farmer, J. J., 3rd. Bacteriophage types of Salmonella typhi in the United States from 1974 through 1981. *J Clin Microbiol* **17**, 172-174, doi:10.1128/jcm.17.1.172-174.1983 (1983).

44 Chattaway, M. A., Langridge, G. C. & Wain, J. Salmonella nomenclature in the genomic era: a time for change. *Sci Rep* **11**, 7494, doi:10.1038/s41598-021-86243-w (2021).

45 Wong, V. K. *et al.* Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter- and intracontinental transmission events. *Nat Genet* **47**, 632-639, doi:10.1038/ng.3281 (2015).

46 Tanmoy, A. M. *et al.* Paratype: a genotyping tool for Salmonella Paratyphi A reveals its global genomic diversity. *Nature Communications* **13**, 7912, doi:10.1038/s41467-022-35587-6 (2022).

47 Kariuki, S. *et al.* Multiple introductions of multidrug-resistant typhoid associated with acute infection and asymptomatic carriage, Kenya. *Elife* **10**, doi:10.7554/eLife.67852 (2021).

48 Dyson, Z. A. *et al.* Genomic epidemiology and antimicrobial resistance transmission of Salmonella Typhi and Paratyphi A at three urban sites in Africa and Asia. *medRxiv*, 2023.2003.2011.23286741, doi:10.1101/2023.03.11.23286741 (2023).

49 Wong, V. K. *et al.* An extended genotyping framework for Salmonella enterica serovar Typhi, the cause of human typhoid. *Nature Communications* **7**, 12827, doi:10.1038/ncomms12827 (2016).

50 Dyson, Z. A. & Holt, K. E. Five Years of GenoTyphi: Updates to the Global Salmonella Typhi Genotyping Framework. *The Journal of Infectious Diseases* **224**, S775-S780, doi:10.1093/infdis/jiab414 (2021).
51 Carey, M. E. *et al.* Global diversity and antimicrobial resistance of typhoid fever pathogens: insights from 13,000 Salmonella Typhi genomes. *medRxiv*, 2022.2012.2028.22283969, doi:10.1101/2022.12.28.22283969 (2022).
52 da Silva, K. E. *et al.* The international and intercontinental spread and expansion of antimicrobial-resistant Salmonella Typhi: a genomic epidemiology study. *Lancet Microbe* **3**, e567-e577, doi:10.1016/s2666-5247(22)00093-3 (2022).
53 Park, S. E. *et al.* The phylogeography and incidence of multi-drug resistant typhoid fever in sub-Saharan Africa. *Nat Commun* **9**, 5094, doi:10.1038/s41467-018-07370-z (2018).
54 Guevara, P. D. *et al.* A genomic snapshot of Salmonella enterica serovar Typhi in Colombia. *PLoS Negl Trop Dis* **15**, e0009755, doi:10.1371/journal.pntd.0009755 (2021).
55 Maes, M. *et al.* Whole genome sequence analysis of Salmonella Typhi provides evidence of phylogenetic linkage between cases of typhoid fever in Santiago, Chile in the 1980s and 2010–2016. *PLOS Neglected Tropical Diseases* **16**, e0010178, doi:10.1371/journal.pntd.0010178 (2022).
56 Sikorski, M. J. *et al.* Persistence of Rare Salmonella Typhi Genotypes Susceptible to First-Line Antibiotics in the Remote Islands of Samoa. *mBio*, e0192022, doi:10.1128/mbio.01920-22 (2022).
57 Davies, M. R. *et al.* Genomic epidemiology of Salmonella Typhi in Central Division, Fiji, 2012 to 2016. *Lancet Reg Health West Pac* **24**, 100488, doi:10.1016/j.lanwpc.2022.100488 (2022).
58 Wirth, T. Massive lineage replacements and cryptic outbreaks of Salmonella Typhi in eastern and southern Africa. *Nat Genet* **47**, 565-567, doi:10.1038/ng.3318 (2015).
59 Gibani, M. M., Britto, C. & Pollard, A. J. Typhoid and paratyphoid fever: a call to action. *Curr Opin Infect Dis*, doi:10.1097/qco.0000000000000479 (2018).
60 McClelland, M. *et al.* Comparison of genome degradation in Paratyphi A and Typhi, human-restricted serovars of Salmonella enterica that cause typhoid. *Nat Genet* **36**, 1268-1274, doi:10.1038/ng1470 (2004).
61 Parkhill, J. *et al.* Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18. *Nature* **413**, 848-852, doi:10.1038/35101607 (2001).
62 Holt, K. E. *et al.* High-throughput bacterial SNP typing identifies distinct clusters of Salmonella Typhi causing typhoid in Nepalese children. *BMC Infect Dis* **10**, 144, doi:10.1186/1471-2334-10-144 (2010).
63 Hiyoshi, H., Tiffany, C. R., Bronner, D. N. & Bäumler, A. J. Typhoidal Salmonella serovars: ecological opportunity and the evolution of a new pathovar. *FEMS Microbiol Rev* **42**, 527-541, doi:10.1093/femsre/fuy024 (2018).
64 Rogers, A. W. L., Tsolis, R. M. & Bäumler, A. J. Salmonella versus the Microbiome. *Microbiol Mol Biol Rev* **85**, doi:10.1128/mmbr.00027-19 (2021).
65 Johnson, R., Mylona, E. & Frankel, G. Typhoidal Salmonella: Distinctive virulence factors and pathogenesis. *Cell Microbiol* **20**, e12939, doi:10.1111/cmi.12939 (2018).

66 Bronner, D. N. *et al.* Genetic Ablation of Butyrate Utilization Attenuates Gastrointestinal Salmonella Disease. *Cell Host Microbe* **23**, 266-273.e264, doi:10.1016/j.chom.2018.01.004 (2018).
67 Winter, S. E. *et al.* The flagellar regulator TviA reduces pyroptosis by Salmonella enterica serovar Typhi. *Infect Immun* **83**, 1546-1555, doi:10.1128/iai.02803-14 (2015).
68 Pickard, D. *et al.* Composition, acquisition, and distribution of the Vi exopolysaccharide-encoding Salmonella enterica pathogenicity island SPI-7. *J Bacteriol* **185**, 5055-5065, doi:10.1128/jb.185.17.5055-5065.2003 (2003).
69 Ménard, S. *et al.* Cross-Talk Between the Intestinal Epithelium and Salmonella Typhimurium. *Front Microbiol* **13**, 906238, doi:10.3389/fmicb.2022.906238 (2022).
70 Dillon, A. & Lo, D. D. M Cells: Intelligent Engineering of Mucosal Immune Surveillance. *Frontiers in Immunology* **10**, doi:10.3389/fimmu.2019.01499 (2019).
71 Watson, K. G. & Holden, D. W. Dynamics of growth and dissemination of Salmonella in vivo. *Cell Microbiol* **12**, 1389-1397, doi:10.1111/j.1462-5822.2010.01511.x (2010).
72 Hart, P. J. *et al.* Differential Killing of Salmonella enterica Serovar Typhi by Antibodies Targeting Vi and Lipopolysaccharide O:9 Antigen. *PLoS One* **11**, e0145945, doi:10.1371/journal.pone.0145945 (2016).
73 Waddington, C. S. *et al.* An outpatient, ambulant-design, controlled human infection model using escalating doses of Salmonella Typhi challenge delivered in sodium bicarbonate solution. *Clin Infect Dis* **58**, 1230-1240, doi:10.1093/cid/ciu078 (2014).
74 Waddington, C. S. *et al.* Advancing the management and control of typhoid fever: a review of the historical role of human challenge studies. *J Infect* **68**, 405-418, doi:10.1016/j.jinf.2014.01.006 (2014).
75 Dobinson, H. C. *et al.* Evaluation of the Clinical and Microbiological Response to Salmonella Paratyphi A Infection in the First Paratyphoid Human Challenge Model. *Clin Infect Dis* **64**, 1066-1073, doi:10.1093/cid/cix042 (2017).
76 Darton, T. C. *et al.* Blood culture-PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia. *J Infect* **74**, 358-366, doi:10.1016/j.jinf.2017.01.006 (2017).
77 Blohmke, C. J. *et al.* Interferon-driven alterations of the host's amino acid metabolism in the pathogenesis of typhoid fever. *The Journal of Experimental Medicine* **213**, 1061-1077, doi:10.1084/jem.20151025 (2016).
78 Darton, T. C. *et al.* Rapidly Escalating Hepcidin and Associated Serum Iron Starvation Are Features of the Acute Response to Typhoid Infection in Humans. *PLoS Negl Trop Dis* **9**, e0004029, doi:10.1371/journal.pntd.0004029 (2015).
79 Darton, T. C. *et al.* Identification of Novel Serodiagnostic Signatures of Typhoid Fever Using a Salmonella Proteome Array. *Front Microbiol* **8**, 1794, doi:10.3389/fmicb.2017.01794 (2017).
80 Blohmke, C. J. *et al.* Diagnostic host gene signature for distinguishing enteric fever from other febrile diseases. *EMBO Mol Med* **11**, e10431, doi:10.15252/emmm.201910431 (2019).

81 Song, J., Gao, X. & Galán, J. E. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. *Nature* **499**, 350-354, doi:10.1038/nature12377 (2013).
82 Del Bel Belluz, L. *et al.* The Typhoid Toxin Promotes Host Survival and the Establishment of a Persistent Asymptomatic Infection. *PLoS Pathog* **12**, e1005528, doi:10.1371/journal.ppat.1005528 (2016).
83 den Bakker, H. C. *et al.* Genome sequencing reveals diversification of virulence factor content and possible host adaptation in distinct subpopulations of Salmonella enterica. *BMC Genomics* **12**, 425, doi:10.1186/1471-2164-12-425 (2011).
84 Rodriguez-Rivera, L. D., Bowen, B. M., den Bakker, H. C., Duhamel, G. E. & Wiedmann, M. Characterization of the cytolethal distending toxin (typhoid toxin) in non-typhoidal Salmonella serovars. *Gut Pathog* **7**, 19, doi:10.1186/s13099-015-0065-1 (2015).
85 Gibani, M. M. *et al.* Investigation of the role of typhoid toxin in acute typhoid fever in a human challenge model. *Nat Med* **25**, 1082-1088, doi:10.1038/s41591-019-0505-4 (2019).
86 Pitzer, V. E. *et al.* Predicting the impact of vaccination on the transmission dynamics of typhoid in South Asia: a mathematical modeling study. *PLoS Negl Trop Dis* **8**, e2642, doi:10.1371/journal.pntd.0002642 (2014).
87 Saul, A., Smith, T. & Maire, N. Stochastic simulation of endemic Salmonella enterica serovar Typhi: the importance of long lasting immunity and the carrier state. *PLoS One* **8**, e74097, doi:10.1371/journal.pone.0074097 (2013).
88 Gibani, M. M. *et al.* The Impact of Vaccination and Prior Exposure on Stool Shedding of Salmonella Typhi and Salmonella Paratyphi in 6 Controlled Human Infection Studies. *Clin Infect Dis* **68**, 1265-1273, doi:10.1093/cid/ciy670 (2019).
89 Juel, H. B. *et al.* Salmonella Typhi Bactericidal Antibodies Reduce Disease Severity but Do Not Protect against Typhoid Fever in a Controlled Human Infection Model. *Front Immunol* **8**, 1916, doi:10.3389/fimmu.2017.01916 (2017).
90 Jones, E. *et al.* A Salmonella Typhi Controlled Human Infection Study for Assessing Correlation between Bactericidal Antibodies and Protection against Infection Induced by Typhoid Vaccination. *Microorganisms* **9**, doi:10.3390/microorganisms9071394 (2021).
91 Jin, C. *et al.* Treatment responses to Azithromycin and Ciprofloxacin in uncomplicated Salmonella Typhi infection: A comparison of Clinical and Microbiological Data from a Controlled Human Infection Model. *PLoS Negl Trop Dis* **13**, e0007955, doi:10.1371/journal.pntd.0007955 (2019).
92 Jin, C. *et al.* Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of Salmonella Typhi: a randomised controlled, phase 2b trial. *Lancet* **390**, 2472-2480, doi:10.1016/S0140-6736(17)32149-9 (2017).
93 Jin, C. *et al.* Vi-specific serological correlates of protection for typhoid fever. *J Exp Med* **218**, doi:10.1084/jem.20201116 (2021).
94 Dahora, L. C. *et al.* IgA and IgG1 Specific to Vi Polysaccharide of Salmonella Typhi Correlate With Protection Status in a Typhoid Fever Controlled Human Infection Model. *Front Immunol* **10**, 2582, doi:10.3389/fimmu.2019.02582 (2019).

95 Cross, D. L. *et al.* Vi-Vaccinations Induce Heterogeneous Plasma Cell Responses That Associate With Protection From Typhoid Fever. *Front Immunol* **11**, 574057, doi:10.3389/fimmu.2020.574057 (2020).
96 Marchello, C. S., Carr, S. D. & Crump, J. A. A Systematic Review on Antimicrobial Resistance among Salmonella Typhi Worldwide. *Am J Trop Med Hyg*, doi:10.4269/ajtmh.20-0258 (2020).
97 Britto, C. D., Wong, V. K., Dougan, G. & Pollard, A. J. A systematic review of antimicrobial resistance in Salmonella enterica serovar Typhi, the etiological agent of typhoid. *PLoS Negl Trop Dis* **12**, e0006779, doi:10.1371/journal.pntd.0006779 (2018).
98 Bowe, F. *et al.* At least four percent of the Salmonella typhimurium genome is required for fatal infection of mice. *Infect Immun* **66**, 3372-3377, doi:10.1128/iai.66.7.3372-3377.1998 (1998).
99 Chau, T. T. *et al.* Antimicrobial drug resistance of Salmonella enterica serovar typhi in asia and molecular mechanism of reduced susceptibility to the fluoroquinolones. *Antimicrobial agents and chemotherapy* **51**, 4315-4323, doi:10.1128/aac.00294-07 (2007).
100 Day, M. R. *et al.* Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of Salmonella enterica serovars Typhi and Paratyphi. *J Antimicrob Chemother* **73**, 365-372, doi:10.1093/jac/dkx379 (2018).
101 Qamar, F. N. *et al.* Antimicrobial Resistance in Typhoidal Salmonella: Surveillance for Enteric Fever in Asia Project, 2016-2019. *Clin Infect Dis* **71**, S276-s284, doi:10.1093/cid/ciaa1323 (2020).
102 Akram, J. *et al.* Extensively Drug-Resistant (XDR) Typhoid: Evolution, Prevention, and Its Management. *Biomed Res Int* **2020**, 6432580, doi:10.1155/2020/6432580 (2020).
103 Posen, H. J. *et al.* Travel-associated extensively drug-resistant typhoid fever: a case series to inform management in non-endemic regions. *J Travel Med* **30**, doi:10.1093/jtm/taac086 (2023).
104 Herdman, M. T. *et al.* Increasingly limited options for the treatment of enteric fever in travellers returning to England, 2014-2019: a cross-sectional analytical study. *J Med Microbiol* **70**, doi:10.1099/jmm.0.001359 (2021).
105 Ashton, P. M. *et al.* The rapid emergence of Salmonella Typhi with decreased ciprofloxacin susceptibility following an increase in ciprofloxacin prescriptions in Blantyre, Malawi. *medRxiv*, 2023.2003.2027.23287794, doi:10.1101/2023.03.27.23287794 (2023).
106 Wijedoru, L., Mallett, S. & Parry, C. M. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. *Cochrane Database Syst Rev* **5**, Cd008892, doi:10.1002/14651858.CD008892.pub2 (2017).
107 Azmatullah, A., Qamar, F. N., Thaver, D., Zaidi, A. K. & Bhutta, Z. A. Systematic review of the global epidemiology, clinical and laboratory profile of enteric fever. *J Glob Health* **5**, 020407, doi:10.7189/jogh.05.020407 (2015).
108 Aiemjoy, K. *et al.* Diagnostic Value of Clinical Features to Distinguish Enteric Fever From Other Febrile Illnesses in Bangladesh, Nepal, and Pakistan. *Clin Infect Dis* **71**, S257-s265, doi:10.1093/cid/ciaa1297 (2020).
109 Andrews, J. R. *et al.* High Rates of Enteric Fever Diagnosis and Lower Burden of Culture-Confirmed Disease in Peri-urban and Rural Nepal. *J Infect Dis* **218**, S214-s221, doi:10.1093/infdis/jix221 (2018).
110 Antillon, M., Saad, N. J., Baker, S., Pollard, A. J. & Pitzer, V. E. The Relationship Between Blood Sample Volume and Diagnostic Sensitivity of

Blood Culture for Typhoid and Paratyphoid Fever: A Systematic Review and Meta-Analysis. *J Infect Dis* **218**, S255-s267, doi:10.1093/infdis/jiy471 (2018).
111 Wain, J. *et al.* Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. *J Clin Microbiol* **36**, 1683-1687, doi:10.1128/jcm.36.6.1683-1687.1998 (1998).
112 Gilman, R. H., Terminel, M., Levine, M. M., Hernandez-Mendoza, P. & Hornick, R. B. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of Salmonella typhi in typhoid fever. *Lancet* **1**, 1211-1213, doi:10.1016/s0140-6736(75)92194-7 (1975).
113 Mogasale, V., Ramani, E., Mogasale, V. V. & Park, J. What proportion of Salmonella Typhi cases are detected by blood culture? A systematic literature review. *Ann Clin Microbiol Antimicrob* **15**, 32, doi:10.1186/s12941-016-0147-z (2016).
114 Wain, J. *et al.* Specimens and culture media for the laboratory diagnosis of typhoid fever. *J Infect Dev Ctries* **2**, 469-474, doi:10.3855/jidc.164 (2008).
115 Neupane, D. P., Dulal, H. P. & Song, J. Enteric Fever Diagnosis: Current Challenges and Future Directions. *Pathogens* **10**, doi:10.3390/pathogens10040410 (2021).
116 Tennant, S. M. *et al.* Detection of Typhoidal and Paratyphoidal Salmonella in Blood by Real-time Polymerase Chain Reaction. *Clin Infect Dis* **61 Suppl 4**, S241-250, doi:10.1093/cid/civ726 (2015).
117 Sapkota, J. *et al.* Comparative Analysis of Commercially Available Typhoid Point-of-Care Tests: Results of a Prospective and Hybrid Retrospective Multicenter Diagnostic Accuracy Study in Kenya and Pakistan. *J Clin Microbiol* **60**, e0100022, doi:10.1128/jcm.01000-22 (2022).
118 Charles, R. C. *et al.* Immunoproteomic analysis of antibody in lymphocyte supernatant in patients with typhoid fever in Bangladesh. *Clin Vaccine Immunol* **21**, 280-285, doi:10.1128/cvi.00661-13 (2014).
119 Liang, L. *et al.* Immune profiling with a Salmonella Typhi antigen microarray identifies new diagnostic biomarkers of human typhoid. *Sci Rep* **3**, 1043, doi:10.1038/srep01043 (2013).
120 Andrews, J. R. *et al.* Plasma Immunoglobulin A Responses Against 2 Salmonella Typhi Antigens Identify Patients With Typhoid Fever. *Clin Infect Dis* **68**, 949-955, doi:10.1093/cid/ciy578 (2019).
121 Näsström, E. *et al.* Reproducible diagnostic metabolites in plasma from typhoid fever patients in Asia and Africa. *eLife* **6**, e15651, doi:10.7554/eLife.15651 (2017).
122 Aiemjoy, K. *et al.* Estimating typhoid incidence from community-based serosurveys: a multicohort study. *Lancet Microbe* **3**, e578-e587, doi:10.1016/s2666-5247(22)00114-8 (2022).
123 Mylona, E. *et al.* The Identification of Enteric Fever-Specific Antigens for Population-Based Serosurveillance. *The Journal of Infectious Diseases*, doi:10.1093/infdis/jiad242 (2023).
124 Parry, C. M., Hien, T. T., Dougan, G., White, N. J. & Farrar, J. J. Typhoid fever. *N Engl J Med* **347**, 1770-1782, doi:10.1056/NEJMra020201 (2002).
125 Stuart, B. M. & Pullen, R. L. Typhoid; clinical analysis of 360 cases. *Arch Intern Med (Chic)* **78**, 629-661, doi:10.1001/archinte.1946.00220060002001 (1946).

126 Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. & Parry, C. M. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. *Clin Microbiol Rev* **28**, 901-937, doi:10.1128/cmr.00002-15 (2015).
127 Hosoglu, S. *et al.* Risk factors for enteric perforation in patients with typhoid Fever. *Am J Epidemiol* **160**, 46-50, doi:10.1093/aje/kwh172 (2004).
128 Birkhold, M. *et al.* Morbidity and mortality of typhoid intestinal perforation among children in sub-Saharan Africa 1995-2019: A scoping review. *World J Surg*, 1-11, doi:10.1007/s00268-020-05567-2 (2020).
129 Neil, K. P. *et al.* A large outbreak of typhoid fever associated with a high rate of intestinal perforation in Kasese District, Uganda, 2008-2009. *Clin Infect Dis* **54**, 1091-1099, doi:10.1093/cid/cis025 (2012).
130 Mogasale, V. *et al.* Case fatality rate and length of hospital stay among patients with typhoid intestinal perforation in developing countries: A systematic literature review. *PLoS ONE* **9**, doi:10.1371/journal.pone.0093784 (2014).
131 Osuntokun, B. O., Bademosi, O., Ogunremi, K. & Wright, S. G. Neuropsychiatric manifestations of typhoid fever in 959 patients. *Arch Neurol* **27**, 7-13, doi:10.1001/archneur.1972.00490130009002 (1972).
132 Lutterloh, E. *et al.* Multidrug-resistant typhoid fever with neurologic findings on the Malawi-Mozambique border. *Clin Infect Dis* **54**, 1100-1106, doi:10.1093/cid/cis012 (2012).
133 Britto, C. D. *et al.* Laboratory and molecular surveillance of paediatric typhoidal Salmonella in Nepal: Antimicrobial resistance and implications for vaccine policy. *PLoS Negl Trop Dis* **12**, e0006408, doi:10.1371/journal.pntd.0006408 (2018).
134 Britto, C., Pollard, A. J., Voysey, M. & Blohmke, C. J. An Appraisal of the Clinical Features of Pediatric Enteric Fever: Systematic Review and Meta-analysis of the Age-Stratified Disease Occurrence. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **64**, 1604-1611, doi:10.1093/cid/cix229 (2017).
135 Cruz Espinoza, L. M. *et al.* Occurrence of Typhoid Fever Complications and Their Relation to Duration of Illness Preceding Hospitalization: A Systematic Literature Review and Meta-analysis. *Clinical Infectious Diseases* **69**, doi:10.1093/cid/ciz477 (2019).
136 Al-Emran, H. M. *et al.* A Multicountry Molecular Analysis of Salmonella enterica Serovar Typhi With Reduced Susceptibility to Ciprofloxacin in Sub-Saharan Africa. *Clin Infect Dis* **62 Suppl 1**, S42-46, doi:10.1093/cid/civ788 (2016).
137 Levine, M. M., Black, R. E. & Lanata, C. Precise estimation of the numbers of chronic carriers of Salmonella typhi in Santiago, Chile, an endemic area. *J Infect Dis* **146**, 724-726, doi:10.1093/infdis/146.6.724 (1982).
138 Khatri, N. S. *et al.* Gallbladder carriage of Salmonella paratyphi A may be an important factor in the increasing incidence of this infection in South Asia. *Ann Intern Med* **150**, 567-568, doi:10.7326/0003-4819-150-8-200904210-00017 (2009).
139 Gonzalez-Escobedo, G., Marshall, J. M. & Gunn, J. S. Chronic and acute infection of the gall bladder by Salmonella Typhi: understanding the carrier state. *Nat Rev Microbiol* **9**, 9-14, doi:10.1038/nrmicro2490 (2011).

140 Crawford, R. W. *et al.* Gallstones play a significant role in Salmonella spp. gallbladder colonization and carriage. *Proc Natl Acad Sci U S A* **107**, 4353-4358, doi:10.1073/pnas.1000862107 (2010).
141 Dongol, S. *et al.* The microbiological and clinical characteristics of invasive salmonella in gallbladders from cholecystectomy patients in kathmandu, Nepal. *PLoS One* **7**, e47342, doi:10.1371/journal.pone.0047342 (2012).
142 Schiøler, H., Christiansen, E. D., Høybye, G., Rasmussen, S. N. & Greibe, J. Biliary calculi in chronic Salmonella carriers and healthy controls: a controlled study. *Scand J Infect Dis* **15**, 17-19, doi:10.3109/inf.1983.15.issue-1.04 (1983).
143 Olsen, S. J. *et al.* Outbreaks of typhoid fever in the United States, 1960-99. *Epidemiol Infect* **130**, 13-21, doi:10.1017/s0950268802007598 (2003).
144 Bokkenheuser, V. DETECTION OF TYPHOID CARRIERS. *Am J Public Health Nations Health* **54**, 477-486, doi:10.2105/ajph.54.3.477 (1964).
145 Avendano, A. *et al.* Duodenal String Cultures: Practicality and Sensitivity for Diagnosing Enteric Fever in Children. *The Journal of Infectious Diseases* **153**, 359-362, doi:10.1093/infdis/153.2.358 (1986).
146 Hoffman, S. A., Sikorski, M. J. & Levine, M. M. Chronic Salmonella Typhi carriage at sites other than the gallbladder. *PLOS Neglected Tropical Diseases* **17**, e0011168, doi:10.1371/journal.pntd.0011168 (2023).
147 Lin, F. Y. *et al.* Restaurant-associated outbreak of typhoid fever in Maryland: identification of carrier facilitated by measurement of serum Vi antibodies. *J Clin Microbiol* **26**, 1194-1197, doi:10.1128/jcm.26.6.1194-1197.1988 (1988).
148 Gupta, A. *et al.* Evaluation of community-based serologic screening for identification of chronic Salmonella typhi carriers in Vietnam. *Int J Infect Dis* **10**, 309-314, doi:10.1016/j.ijid.2005.06.005 (2006).
149 Ferreccio, C. *et al.* [The detection of chronic Salmonella typhi carriers: a practical method applied to food handlers]. *Rev Med Chil* **118**, 33-37 (1990).
150 Lanata, C. F. *et al.* Vi serology in detection of chronic Salmonella typhi carriers in an endemic area. *Lancet* **2**, 441-443, doi:10.1016/s0140-6736(83)90401-4 (1983).
151 Thompson, L. J. *et al.* Transcriptional response in the peripheral blood of patients infected with Salmonella enterica serovar Typhi. *Proc Natl Acad Sci U S A* **106**, 22433-22438, doi:10.1073/pnas.0912386106 (2009).
152 Tran Vu Thieu, N. *et al.* An evaluation of purified Salmonella Typhi protein antigens for the serological diagnosis of acute typhoid fever. *J Infect* **75**, 104-114, doi:10.1016/j.jinf.2017.05.007 (2017).
153 Näsström, E. *et al.* Diagnostic metabolite biomarkers of chronic typhoid carriage. *PLoS Negl Trop Dis* **12**, e0006215, doi:10.1371/journal.pntd.0006215 (2018).
154 Hejfec, L. B. RESULTS OF THE STUDY OF TYPHOID VACCINES IN FOUR CONTROLLED FIELD TRIALS IN THE USSR. *Bull World Health Organ* **32**, 1-14 (1965).
155 World Health Organization. Typhoid vaccines: WHO position paper. *Wkly Epidemiol Rec* **83**, 49-59 (2008).
156 Engels, E. A., Falagas, M. E., Lau, J. & Bennish, M. L. Typhoid fever vaccines: a meta-analysis of studies on efficacy and toxicity. *Bmj* **316**, 110-116, doi:10.1136/bmj.316.7125.110 (1998).

157 Milligan, R., Paul, M., Richardson, M. & Neuberger, A. Vaccines for preventing typhoid fever. *Cochrane Database Syst Rev* **5**, Cd001261, doi:10.1002/14651858.CD001261.pub4 (2018).
158 Goldblatt, D. Conjugate vaccines. *Clin Exp Immunol* **119**, 1-3, doi:10.1046/j.1365-2249.2000.01109.x (2000).
159 World Health Organization. Typhoid vaccines: WHO position paper – March 2018. 153-172 (Geneva, Switzerland, 2018).
160 Mohan, V. K. *et al.* Safety and immunogenicity of a Vi polysaccharide-tetanus toxoid conjugate vaccine (Typbar-TCV) in healthy infants, children, and adults in typhoid endemic areas: a multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study. *Clin Infect Dis* **61**, 393-402, doi:10.1093/cid/civ295 (2015).
161 Meiring, J. E., Giubilini, A., Savulescu, J., Pitzer, V. E. & Pollard, A. J. Generating the Evidence for Typhoid Vaccine Introduction: Considerations for Global Disease Burden Estimates and Vaccine Testing Through Human Challenge. *Clin Infect Dis* **69**, S402-s407, doi:10.1093/cid/ciz630 (2019).
162 Nampota, N. *et al.* Safety and immunogenicity of a typhoid conjugate vaccine among children aged 9 months through 12 years in Malawi: results from a randomised, double-blind, controlled trial *Lancet Glob Health* (2022).
163 Sirima, S. B. *et al.* Safety and immunogenicity of co-administration of meningococcal type A and measles-rubella vaccines with typhoid conjugate vaccine in children aged 15-23 months in Burkina Faso. *Int J Infect Dis* **102**, 517-523, doi:10.1016/j.ijid.2020.10.103 (2021).
164 Meiring, J. E. *et al.* Typhoid Vaccine Acceleration Consortium Malawi: A phase III, randomized, double-blind, controlled trial of the clinical efficacy of typhoid conjugate vaccine among children in Blantyre, Malawi. *Clin Infect Dis* **68**, S50-s58, doi:10.1093/cid/ciy1103 (2019).
165 Patel, P. D. *et al.* Safety and Efficacy of a Typhoid Conjugate Vaccine in Malawian Children. *N Engl J Med* **385**, 1104-1115, doi:10.1056/NEJMoa2035916 (2021).
166 Colin-Jones, R. *et al.* Logistics of Implementing a Large-scale Typhoid Vaccine Trial in Kathmandu, Nepal. *Clin Infect Dis* **68**, S138-s145, doi:10.1093/cid/ciy1125 (2019).
167 Shakya, M. *et al.* Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. *N Engl J Med* **381**, 2209-2218, doi:10.1056/NEJMoa1905047 (2019).
168 Theiss-Nyland, K. *et al.* Assessing the impact of a Vi-polysaccharide conjugate vaccine in preventing typhoid infection among Bangladeshi children: A protocol for a phase IIIb trial. *Clin Infect Dis* **68**, S74-s82, doi:10.1093/cid/ciy1107 (2019).
169 Qadri, F. *et al.* Protection by vaccination of children against typhoid fever with a Vi-tetanus toxoid conjugate vaccine in urban Bangladesh: a cluster-randomised trial. *Lancet* **398**, 675-684, doi:10.1016/s0140-6736(21)01124-7 (2021).
170 Khanam, F. *et al.* Assessment of vaccine herd protection in a cluster-randomised trial of Vi conjugate vaccine against typhoid fever: results of further analysis. *eClinicalMedicine* **58**, doi:10.1016/j.eclinm.2023.101925 (2023).

171 Hoffman, S. A. *et al.* Programmatic Effectiveness of a Pediatric Typhoid Conjugate Vaccine Campaign in Navi Mumbai, India. *Clinical Infectious Diseases*, doi:10.1093/cid/ciad132 (2023).
172 Yousafzai, M. T. *et al.* Effectiveness of typhoid conjugate vaccine against culture-confirmed Salmonella enterica serotype Typhi in an extensively drug-resistant outbreak setting of Hyderabad, Pakistan: a cohort study. *Lancet Glob Health* **9**, e1154-e1162, doi:10.1016/s2214-109x(21)00255-2 (2021).
173 Lightowler, M. S. *et al.* Effectiveness of typhoid conjugate vaccine in Zimbabwe used in response to an outbreak among children and young adults: A matched case control study. *Vaccine* **40**, 4199-4210, doi:10.1016/j.vaccine.2022.04.093 (2022).
174 Nampota-Nkomba, N., Carey, M. E., Jamka, L. P., Fecteau, N. & Neuzil, K. M. Using Typhoid Conjugate Vaccines to Prevent Disease, Promote Health Equity, and Counter Drug-Resistant Typhoid Fever. *Open Forum Infectious Diseases* **10**, S6-S12, doi:10.1093/ofid/ofad022 (2023).
175 Khanam, F., Ross, A. G., McMillan, N. A. J. & Qadri, F. Toward Typhoid Fever Elimination. *Int J Infect Dis* **119**, 41-43, doi:10.1016/j.ijid.2022.03.036 (2022).
176 Phillips, M. T., Owers, K. A., Grenfell, B. T. & Pitzer, V. E. Changes in historical typhoid transmission across 16 U.S. cities, 1889-1931: Quantifying the impact of investments in water and sewer infrastructures. *PLoS Negl Trop Dis* **14**, e0008048, doi:10.1371/journal.pntd.0008048 (2020).
177 Nga, T. V. T., Duy, P. T., Lan, N. P. H., Chau, N. V. V. & Baker, S. The Control of Typhoid Fever in Vietnam. *Am J Trop Med Hyg* **99**, 72-78, doi:10.4269/ajtmh.18-0035 (2018).
178 Browne, A. J. *et al.* Drug-resistant enteric fever worldwide, 1990 to 2018: a systematic review and meta-analysis. *BMC Med* **18**, 1, doi:10.1186/s12916-019-1443-1 (2020).
179 Bhutta, Z. A. Impact of age and drug resistance on mortality in typhoid fever. *Arch Dis Child* **75**, 214-217, doi:10.1136/adc.75.3.214 (1996).
180 Parry, C. M. The treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever in Viet Nam. *Trans R Soc Trop Med Hyg* **98**, 413-422, doi:10.1016/j.trstmh.2003.10.014 (2004).
181 Nabarro, L. E. *et al.* British infection association guidelines for the diagnosis and management of enteric fever in England. *J Infect* **84**, 469-489, doi:10.1016/j.jinf.2022.01.014 (2022).
182 Dolecek C, P. S., Basnyat B, Olliaro P. . *Antibiotics for typhoid fever. In: The selection and use of essential medicines. WHO technical report series No 1021*, <https://apps.who.int/iris/bitstream/handle/10665/330668/9789241210300-eng.pdf?ua=1> (2019).
183 Carey, M. E. *et al.* Spontaneous Emergence of Azithromycin Resistance in Independent Lineages of Salmonella Typhi in Northern India. *Clin Infect Dis* **72**, e120-e127, doi:10.1093/cid/ciaa1773 (2021).
184 Duy, P. T. *et al.* The emergence of azithromycin-resistant Salmonella Typhi in Nepal. *JAC Antimicrob Resist* **2**, dlaa109, doi:10.1093/jacamr/dlaa109 (2020).
185 Effa, E. E. & Bukirwa, H. Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst Rev*, Cd006083, doi:10.1002/14651858.CD006083.pub2 (2008).

186 Effa, E. E. *et al.* Fluoroquinolones for treating typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst Rev* **2011**, Cd004530, doi:10.1002/14651858.CD004530.pub4 (2011).
187 Eliakim-Raz, N. *et al.* Efficacy and safety of chloramphenicol: joining the revival of old antibiotics? Systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* **70**, 979-996, doi:10.1093/jac/dku530 (2015).
188 Qamar, F. N. *et al.* Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. *Lancet Infect Dis* **18**, 1368-1376, doi:10.1016/s1473-3099(18)30483-3 (2018).
189 Klemm, E. *et al.* Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins *MBio* **9**, 1-10 (2018).
190 Argimón, S. *et al.* Circulation of Third-Generation Cephalosporin Resistant Salmonella Typhi in Mumbai, India. *Clin Infect Dis* **74**, 2234-2237, doi:10.1093/cid/ciab897 (2022).
191 Samajpati, S., Pragasam, A. K., Mandal, S., Balaji, V. & Dutta, S. Emergence of ceftriaxone resistant Salmonella enterica serovar Typhi in Eastern India. *Infection, Genetics and Evolution* **96**, 105093, doi:https://doi.org/10.1016/j.meegid.2021.105093 (2021).
192 Qureshi, S. *et al.* Response of extensively drug resistant Salmonella Typhi to treatment with meropenem and azithromycin, in Pakistan. *PLOS Neglected Tropical Diseases* **14**, e0008682, doi:10.1371/journal.pntd.0008682 (2020).
193 Van Be Bay, P. *et al.* Quantitative bacterial counts in the bone marrow of Vietnamese patients with typhoid fever. *Trans R Soc Trop Med Hyg* **116**, 736-744, doi:10.1093/trstmh/trac003 (2022).
194 Pascual, A., Conejo, M. C., García, I. & Perea, E. J. Factors affecting the intracellular accumulation and activity of azithromycin. *J Antimicrob Chemother* **35**, 85-93, doi:10.1093/jac/35.1.85 (1995).
195 Zmora, N. *et al.* Open label comparative trial of mono versus dual antibiotic therapy for Typhoid Fever in adults. *PLoS Negl Trop Dis* **12**, e0006380, doi:10.1371/journal.pntd.0006380 (2018).
196 Giri, A. *et al.* Azithromycin and cefixime combination versus azithromycin alone for the out-patient treatment of clinically suspected or confirmed uncomplicated typhoid fever in South Asia: a randomised controlled trial protocol. *Wellcome Open Res* **6**, 207, doi:10.12688/wellcomeopenres.16801.2 (2021).
197 Butler, T. *et al.* Typhoid fever complicated by intestinal perforation: a persisting fatal disease requiring surgical management. *Rev Infect Dis* **7**, 244-256, doi:10.1093/clinids/7.2.244 (1985).
198 Hoffman, S. L. *et al.* Reduction of mortality in chloramphenicol-treated severe typhoid fever by high-dose dexamethasone. *N Engl J Med* **310**, 82-88, doi:10.1056/nejm198401123100203 (1984).
199 Contopoulos-Ioannidis, D. G. & Ioannidis, J. P. Claims for improved survival from systemic corticosteroids in diverse conditions: an umbrella review. *Eur J Clin Invest* **42**, 233-244, doi:10.1111/j.1365-2362.2011.02584.x (2012).

200 Gal-Mor, O. Persistent Infection and Long-Term Carriage of Typhoidal and Nontyphoidal Salmonellae. *Clin Microbiol Rev* **32**, doi:10.1128/cmr.00088-18 (2019).
201 McCann, N., Scott, P., Parry, C. M. & Brown, M. Antimicrobial agents for the treatment of enteric fever chronic carriage: A systematic review. *PLoS One* **17**, e0272043, doi:10.1371/journal.pone.0272043 (2022).
202 Luthra, K. *et al.* A Review of the Economic Evidence of Typhoid Fever and Typhoid Vaccines. *Clin Infect Dis* **68**, S83-s95, doi:10.1093/cid/ciy1122 (2019).
203 Riewpaiboon, A. *et al.* Cost of illness due to typhoid Fever in pemba, zanzibar, East Africa. *J Health Popul Nutr* **32**, 377-385 (2014).
204 Bahl, R. *et al.* Costs of illness due to typhoid fever in an Indian urban slum community: implications for vaccination policy. *J Health Popul Nutr* **22**, 304-310 (2004).
205 Kaljee, L. M. *et al.* Social and Economic Burden Associated With Typhoid Fever in Kathmandu and Surrounding Areas: A Qualitative Study. *J Infect Dis* **218**, S243-s249, doi:10.1093/infdis/jix122 (2018).
206 Limani, F. *et al.* Estimating the economic burden of typhoid in children and adults in Blantyre, Malawi: a costing cohort study. In progress. *BMC Public Health* (Submitted).
207 Mejia, N. *et al.* Typhoid and Paratyphoid Cost of Illness in Nepal: Patient and Health Facility Costs From the Surveillance for Enteric Fever in Asia Project II. *Clin Infect Dis* **71**, S306-s318, doi:10.1093/cid/ciaa1335 (2020).
208 Mejia, N. *et al.* Typhoid and Paratyphoid Cost of Illness in Bangladesh: Patient and Health Facility Costs From the Surveillance for Enteric Fever in Asia Project II. *Clin Infect Dis* **71**, S293-s305, doi:10.1093/cid/ciaa1334 (2020).
209 Mejia, N. *et al.* Typhoid and Paratyphoid Cost of Illness in Bangladesh: Patient and Health Facility Costs From the Surveillance for Enteric Fever in Asia Project II. *Clinical Infectious Diseases* **71**, S293-S305, doi:10.1093/cid/ciaa1334 (2020).
210 Mejia, N. *et al.* Typhoid and Paratyphoid Cost of Illness in Pakistan: Patient and Health Facility Costs From the Surveillance for Enteric Fever in Asia Project II. *Clin Infect Dis* **71**, S319-s335, doi:10.1093/cid/ciaa1336 (2020).
211 Bilcke, J. *et al.* Cost-effectiveness of routine and campaign use of typhoid Vi-conjugate vaccine in Gavi-eligible countries: a modelling study. *Lancet Infect Dis* **19**, 728-739, doi:10.1016/s1473-3099(18)30804-1 (2019).
212 Seyi-Olajide, J. O. *et al.* Catastrophic Healthcare Expenditure from Typhoid Perforation in Children in Nigeria. *Surg Infect (Larchmt)* **21**, 586-591, doi:10.1089/sur.2020.134 (2020).
213 Kumar, D. *et al.* Cost of Illness Due to Severe Enteric Fever in India. *J Infect Dis* **224**, S540-s547, doi:10.1093/infdis/jiab282 (2021).
214 Malik, A. (2019).
215 Limani, F. *et al.* Estimating the economic burden of typhoid in children and adults in Blantyre, Malawi: A costing cohort study. *PLOS ONE* **17**, e0277419, doi:10.1371/journal.pone.0277419 (2022).
216 Birkhold, M., Mwisongo, A., Pollard, A. J. & Neuzil, K. M. Typhoid conjugate vaccine in Africa and Asia: Status of clinical evaluation and vaccine introduction. *The Journal of Infectious Diseases*, doi:10.1093/infdis/jiab449 (2021).

217 World Health, O. in *Vaccine* Vol. 37 (2019).
218 World Health Organization. Weekly epidemiological record. 261-276 (Geneva, Switzerland, 2022).
219 Gavi the Vaccine Alliance. (2017).
220 Lo, N. C. *et al.* Comparison of Strategies and Incidence Thresholds for Vi Conjugate Vaccines Against Typhoid Fever: A Cost-effectiveness Modeling Study. *J Infect Dis* **218**, S232-s242, doi:10.1093/infdis/jix598 (2018).
221 Burrows, H. *et al.* Comparison of model predictions of typhoid conjugate vaccine public health impact and cost-effectiveness. *Vaccine* **41**, 965-975, doi:10.1016/j.vaccine.2022.12.032 (2023).
222 Chauhan, A. S. *et al.* Cost effectiveness of typhoid vaccination in India. *Vaccine* **39**, 4089-4098, doi:10.1016/j.vaccine.2021.06.003 (2021).
223 Ryckman, T. *et al.* Comparison of Strategies for Typhoid Conjugate Vaccine Introduction in India: A Cost-Effectiveness Modeling Study. *The Journal of Infectious Diseases* **224**, S612-S624, doi:10.1093/infdis/jiab150 (2021).
224 Shakya, M. *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. *Lancet Glob Health* **9**, e1561-e1568, doi:10.1016/s2214-109x(21)00346-6 (2021).
225 Batool, R. *et al.* Effectiveness of typhoid conjugate vaccine against culture-confirmed typhoid in a peri-urban setting in Karachi: A case-control study. *Vaccine* **39**, 5858-5865, doi:10.1016/j.vaccine.2021.08.051 (2021).