Chapter

Enteric Fever in Primary Care

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Abstract

Enteric fever is a bacterial infection caused by *Salmonella typhi* and *paratyphi*. It is endemic in many parts of Africa and South Asia where there is poor access to safe portable water and below par food quality assurance. It is important to ensure prompt recognition, diagnosis and management of symptoms to forestall complications. Due to the rising global burden, significant effort has to be made to improve primary care services like vaccination, antimicrobial stewardship and encouragement of hygiene measures. Hence, it is imperative to be aware of its current burden and options available in primary care for its prevention and treatment.

Keywords: *Salmonella*, enteric fever, typhoid, primary care, typhoid-conjugate vaccine

1. Introduction

Typhoid fever (now more appropriately called Enteric fever) is a bacterial infectious disease caused by *Salmonella enterica* subspecies *enterica* and serovar *typhi*. It is mainly transmitted through the faeco-oral route via contaminated food, water and asymptomatic carriers [1]. It is endemic in developing countries and low-resource settings where hygiene and sanitation measures are subpar. In developed countries and high-income settings, it is less common but cases still occur in recent travellers to endemic areas [2]. There are a number of factors which contribute to the disease burden including lack of access to clean, portable water, poor food quality control and lack of public health services (e.g well managed public latrine and hand washing facilities); all of which can be attributed to lack of awareness, low political will and sociocultural factors. Symptoms of enteric fever vary significantly and are generally nonspecific. These include pyrexia, headache, myalgia, arthralgia, nausea, rash, abdominal pain, constipation and occasionally diarrhoea [3].

Enteric fever, if left untreated can be life-threatening and result in a myriad of complications including intestinal haemorrhage and perforation, peritonitis, sepsis, meningitis, osteomyelitis, multiorgan failure and death [1, 3]. Hence, it is expedient to ensure early diagnosis and management to mitigate complications.

Central to the actualisation of universal health coverage is an effective primary health care system which is usually the first point of contact for most patients. Hence, the role of the primary care clinician in the prevention, diagnosis and management of enteric fever and its complications cannot be overemphasised. This is what this chapter aims to address.

2. Epidemiology

Enteric fever is a global health problem affecting 21.6 million people (incidence of 3.6 per 1000 population) and resulting in just over 216 000 deaths annually [4]. It is endemic in developing and low and middle income countries of Africa, Asia, Latin America, the Caribbean and Oceania mainly due to poor sanitation and environmental hygiene [2, 4]. Bangladesh, Indonesia, China, India, Laos, Nepal, Pakistan and Vietnam account for 80% of cases [4]. Untreated, 10%–30% of patients will die but mortality reduces to 1%–4% with prompt and appropriate treatment. In the pre-antibiotics era, the USA had a case fatality rate of 9%–13% [5]. This illustrates how much the discovery of antibiotics has revolutionised its management, just like most bacterial infections. However, there is an increasing burden of antimicrobial resistant *Salmonella* strains emanating from endemic countries mainly due to poor antimicrobial stewardship and measures must be taken to stem the tide.

Significant intra- and intercountry variation in disease burden exists in many regions of south and south east Asia and parts of Africa. For instance, surveillance performed in two sites in Kenya between 2006 and 2009 found that the incidence of blood-culture proven typhoid fever in rural and urban sites varied from 29 up to 247-cases/100 000 person-years [6]. Also, data from the Diseases of Most Impoverished areas have described incidence rates varying from 24.2/100 000 in Vietnam to 493.5/100 000 in parts of India [7]. However, most disease burden data from low- and middle-income countries are hospital-based which leaves a huge number of cases unaccounted especially in areas of low health-care usage and accessibility. Hence, it is imperative for countries in endemic regions of the world to develop a national and regional surveillance system to identify factors responsible for these variations and adopt guidelines and protocols to improve efficiency in prevention, diagnosis and management. Central to this should be an efficient primary care system where surveillance and data gathering can be co-ordinated and synchronised with hospital-based data providing a broad-based approach and a better reflection of disease burden.

The incidence of enteric fever varies by age. In endemic areas, incidence is higher in younger children but similar across age groups in low burden areas [8]. In general, children are at a higher risk of complications including ileitis and intestinal perforation. When perforation sets in, mortality has been reported to be as high as 62% [9, 10]. Therefore, it is imperative that signs and symptoms of enteric fever are identified and treated early in primary care. Due to the wide disparity in incidence between developed and low and middle-income countries, primary care physicians in the latter will most likely see a lot more cases and have a high pre-test probability. This poses a challenge for a lot of primary care practitioners in developed countries who are less likely to be familiar with its presentation and may result in delay in diagnosis. In England and Wales, any case of *Salmonella* infection is a notifiable disease which must be reported to Public Health England and may require urgent community investigation to forestall an outbreak [11].

3. Aeotiopathogenesis

Salmonella is a flagellated, non-capsulated facultative anaerobic gram-negative bacilli and non-lactose fermenter of the Enterobacteriaceae family which has flagellar, somatic and outer coat antigens [7, 12]. Its outermost covering is made up of the somatic O antigen while the flagellae are composed of the H antigen. Each

O and H antigen have a unique code number and a varied combination of these form the basis for the determination of serotypes [12]. Of the over 2500 serotypes of *Salmonella* that have been identified, only 100 are thought to be responsible for most human infections [7]. These infections can be broadly divided into nontyphoidal and typhoidal. The typhoidal infection is mainly caused by *S. typhi* and less commonly *paratyphi*. *Salmonella typhi* and *paratyphi* A are thought to be restricted to humans alone. A key virulence factor in most strains of *S.typhi* is the Vi capsular antigen which possesses immunomodulatory properties that are thought to contribute to disease pathogenesis, including limiting complement deposition, reducing immune activation, assisting with phagocytosis evasion, and inhibiting serum bactericidal activity [7, 13, 14]. Without it, *S. typhi* will be more susceptible to attack and destruction by the host immune system. Hence, the Vi antigen has been harnessed as a major component of typhoid vaccines including the new conjugate vaccines [7].

Transmission is through the faeco-oral route from contaminated food, water and unrestricted contact with chronic carriers especially in an unhygienic environment. When *Salmonella typhi* is ingested, it evades degradation by enzymes and gastric acid before entering the host's system primarily through the terminal ileum [15]. At the distal ileum, through specialised structures called fimbrae, they attach to the epithelial cells overlying clusters of lymphoid tissues called Peyer patches. These serve as a relay point for macrophages travelling from the gut to the lymphatic system. Activation of the macrophages at the Peyer's patches release cytokines which attract more macrophages to the site. These macrophages serve as a vehicle by which S. typhi is transported to several parts of the reticuloendothelial system including the liver, spleen and bone marrow where they replicate up to a critical density at this point [16], they break into the bloodstream and invade other parts of the body. One of such places invaded is the gall bladder. The gall bladder is infected haematogenously or through infected bile. Infected bile is then secreted into the gut where it once again comes in contact with the Peyer patches at the distal ileum. This second sensitization of the macrophages at this site results in inflammation and hypertrophy of the lymphoid tissues (typhoid ileitis) [15, 16]. This enlargement encroaches on the blood supply resulting in ischaemic coagulative necrosis and consequently perforation and peritonitis. Some of the *salmonella* is excreted in the stool which is serves as a source of infection spread. This is the source of transmission of *Salmonella* in chronic carriers where the *salmonella* is thought to avoid enzymatic and chemical degradation in the gall bladder for a long time by forming biofilms or entering an intracellular 'comfort zone' in the gall bladder epithelium.

4. Clinical presentation

Enteric fever presents with a number of nonspecific symptoms and a wide variation in severity. Symptoms must be correlated with laboratory investigation to reach a diagnosis. Symptoms generally include fever, constipation, diarrhoea, abdominal pain, lethargy, nausea and vomiting, malaise, headache, truncal rash (rose spot), anorexia etc. The incubation period for enteric fever is 1–3 weeks and symptoms progressively get worse over the course of illness if not promptly treated [17].

In the first week, patients may complain of headache, malaise, intermittent fever, cough and constipation. Bradycardia may also be elicited on clinical examination. In the presence of fever, this is termed Faget sign or sphygmothermic dissociation. This is also seen in yellow fever, Brucellosis, Tularaemia and Colorado Tick fever.

In the second week, the patient appears dull with diarrhoea and apathy, sustained pyrexia, distended, tender abdomen and sometimes red macules (rose spots). Splenomegaly may also be present in 75% of cases.

In the third week if still untreated, patient become very ill, delirious and toxic with high pyrexia, intestinal haemorrhage and perforation. Toxic myocarditis may also ensue.

10% of cases relapse within the first 3 weeks of apparent recovery or completion of treatment, hence adequate monitoring and follow up should be arranged.

In the United Kingdom, any *Salmonella* infection is notifiable to Public Health England. Most cases occur in travellers returning from endemic areas. The PHE has developed certain criteria which would serve as an invaluable tool especially for primary care physicians in the early identification of suspicious cases for further escalation, assessment and confirmation.

According to Public Health England (PHE), cases can be classified into confirmed, possible and probable cases based on the following criteria (**Table 1**) [18]:

Confirmed Case	Probable Case	Possible Case
• A person *with S. typhi* or *S. paratyphi* infection determined by the Public Health England Gastrointestinal Bacteria Reference Unit OR • A person with documented confirmatory evidence from a recognised overseas reference laboratory	• Local laboratory presumptive identification of *Salmonella typhi* or *paratyphi* on faecal and/or blood culture or culture of another sterile site (e.g. urine), with or without clinical history compatible with enteric fever OR • A returning traveller giving a clinical history compatible with enteric fever and documentation of a positive blood/faecal culture (or positive PCR for *S. typhi* / *S. paratyphi* on blood) and/or treatment for enteric fever overseas	• A person with a clinical history compatible with enteric fever and where the clinician suspects typhoid or paratyphoid as the most likely diagnosis OR • A person with clinical history of fever and malaise and/or gastrointestinal symptoms with an epidemiological link to a source of enteric fever e.g. if they have 'Warn and inform' information OR • A returning traveller reporting a diagnosis abroad with positive serological testing or *Salmonella* PCR from faeces but no documented evidence of a positive blood or faecal culture positive

Table 1.
PHE classification of Enteric fever cases

It must be noted that the typical presentation of course of enteric fever may deviate significantly from that described above. These may include pneumonia, delirium, arthralgias and severe jaundice. Younger children, people living with AIDS and one third of immunocompetent adults may present with diarrhoea instead of the classical constipation. The typical step ladder pyrexia is now only seen I 12% of cases with the fever pattern now mostly of the insidious persistent type [7]. Untreated or poorly treated infections may result in orchitis, intestinal ileitis, haemorrhage and perforation, meningitis, osteomyelitis.

5. Diagnosis

The diagnosis of enteric fever is made by correlation of clinical and laboratory investigations. The current gold standard as recommended by the world health

organisation (WHO) is blood culture, although this may be culture of bone marrow, stool or urine depending on the time in the course of infection at which the sample was taken [4, 7, 15]. Even blood culture has been found to be an imperfect gold standard, hence there is an advocacy in some quarters for the use of a composite reference standard (CRS) to improve estimation of diagnostic accuracy [19]. The CRS involves combination of several diagnostic tests to increase the sensitivity rather than relying on individual tests. However, at present there is no consensus as to which tests should be included in the CRS [19]. This may be the future gold standard but further research is needed.

In low- and middle-income countries where the disease is endemic, access to contemporary diagnostic tests may be a challenge and a lot of patient in these countries pay out of pocket for health service delivery which they may not be able to afford. Hence, there is a case for empirical treatment based on clinical symptoms. However, this should be seen as a last resort and priority should be given to improving access to a simple, effective rapid diagnostic test (RDT) which is both reliable and valid. At present, although there are RDTs available commercially in endemic areas such as Typhidot, TUBEX and Test-it, their diagnostic accuracy is uncertain [20].

A lot of laboratories in low resource settings are still very much dependent on the Widal test. The Widal test is a serologic agglutination test developed by F Widal in 1896 [21]. The test is based on the presence of antibodies against the flagellar H and somatic O antigens of *Salmonella* typhi. Over the years, it has become a lot more controversial and largely abandoned in developed countries [21]. The main limitations with the test include a high cross- reactivity with other infectious agents (like nontyphoidal *salmonella*, plasmodium and tuberculosis), past enteric fever and BCG vaccination history. Other limitations include poor performance technique and result interpretation. Therefore, its use should be restricted to situations where there is no other supportive confirmatory test [21].

There are lots of other tests in development which hold promise for the future of enteric fever diagnosis. The antibody-in lymphocyte-supernatant (ALS) test has demonstrated good sensitivity and specificity in endemic settings [7, 22]. Others include PCR-based assays and high through-put technologies on clinical specimens using mass spectrometry [23].

6. Clinical management

In primary care, a thorough history and clinical examination could be suggestive. Unstable patients or those at high risk of deterioration should be referred to secondary care for same day hospital assessment and treatment. The mainstay of enteric fever treatment is antibiotics. The route of administration is often oral and parenteral in primary and secondary care respectively. There are several options of antibiotics and first line choice is usually determined by national and local guidelines according to sensitivities and antibiotics resistance pattern. In most places, fluoroquinolones such as ciprofloxacin and ofloxacin are first line. Second line antibiotics include third generation cephalosporins such as ceftriaxone, ampicillin, co-amoxiclav and trimethoprim-sulphamethoxazole [7]. Over the years, several options of antibiotics have been preferred but have changed based on resistance patterns. Decades ago, top on the list of antibiotics were chloramphenicol, ampicillin and co-trimoxazole [7]. The resistance to these traditional antibiotics resulted in multidrug resistance (MDR) typically conferred via IncHI1 plasmids, harbouring resistance genes such as *catA*, *sul1*, *sul2*, *dfrA*, $bla_{\text{TEM-1}}$, *strA*, *strB*, *tetA*, *tetB*, *tetC,* and *tetD* on composite transposons [7]. MDR strains were responsible for several

outbreaks of enteric fever in the 1980/1990s and led to the widespread use of fluoroquinolones as first-line therapy [7, 24]. In the event of MDR and fluoroquinolone resistance, third generation cephalosporins provided respite and an effective alternative. Unfortunately, there are now emerging resistant strains to fluroquinolone and cephalosporin especially in Africa, south-east Asia and the Indian subcontinent resulting in extreme drug-resistance [7]. Fluoroquinolone resistance occurs mainly via chromosomal mutations in the *gyrA*, *gyrB*, *parC,* and *parE* genes. The local pharmacologist or microbiologist should be involved in discussion of the treatment of such cases where recommendations can be made for the use of other options. Such options would likely include Azithromycin, tigecycline or the monobactam, Aztreonam. On a positive note, re-emerging sensitivity to the traditional antibiotics of chloramphenicol, ampicillin and co-trimoxazole is being reported after the prolonged decline in their use [25]. This makes a case for strict adherence to antibiotics stewardship and similar trend may be the case for lot of other infectious diseases which is worth exploring. It is inevitable that various forms of MDR may emerge in future and antibiotic guidelines have to evolve to reflect the trend.

Also, chronic carriers may be treated with a combination of medical and surgical interventions. About 80% clearance rate can be achieved with a 28- day course of ciprofloxacin 750 mg twice daily or norfloxacin 400 mg twice daily. Azithromycin may be beneficial in those with floroquinolone resistance. In chronic carriers with cholethiasis, cholecystectomy under antibiotic cover is indicated and those with schistosomal infection should be covered with praziquantel [7, 24].

7. Prevention

As earlier discussed, central to the transmission and pathogenesis of enteric fever is poor sanitation and hygiene standards and lack of access to safe drinking water. Therefore, public health interventions targeted at addressing these will go a long way in reducing the global burden of the disease.

Another factor responsible for high global burden is the emergence of antimicrobial resistance strains which result in treatment failure and increased carriage rate [7, 14, 16]. In light of this, it is totally rational that the development of a highly efficacious vaccine will significantly reduce global burden. This is highly important especially for endemic areas with high disease burden and those at high risk of complications especially young children. A number of typhoid vaccines have been developed over the years and others are still in various stages of development with varying degrees of efficacy. Examples include Ty21a and Vi-polysaccharide vaccines which have shown efficacy at 2 years of 58% (95% CI 40–71%) and 59% (95% CI 45–69%), respectively [7]. Typhoid conjugate vaccines (TCVs) have been developed using the Vi-polysaccharide vaccine covalently linked to a protein to enhance immunogenicity, antibody quality, magnitude and duration [7]. Recent studies have shown better and long-lasting immunogenicity from TCV than Vi alone. For instance a prototype Vi-rEPA vaccine made up of Vi covalently linked to rEPA, a recombinant exoprotein A from *Pseudomonas aeruginosa* demonstrated efficacy of up to 91% (95% CI 77–97%) at 2 years, when given as a two dose schedule in 2–5 year-old children and protection lasted at least 4 years [7, 26, 27].

In England, the two main vaccines available are the Vi vaccine given as a single injection and the Ty21a vaccine available in the form of three capsules to be taken on alternate days. It is also available in combination with hepatitis A vaccine with protection lasting 1 year and 3 years for hepatitis A and Typhoid respectively [28]. Vaccination is highly recommended for people who are travelling to high risk areas including the Indian subcontinent, Africa, South America, South and South-east

Asia [28]. The Ty21a being live-attenuated, should not be given to immunocompromised patients or children below six years of age. However, the Vi vaccine can be given from the age of 2 years [28].

The World Health Organisation (WHO) strategic Group of Experts on immunisation, in October, 2017, recommended the inclusion of TCVs in vaccination programme schedules in endemic countries from 6 month of age and catch-up vaccinations in children and adolescents up to 15 years old where it is feasible and appropriate [7, 29]. However, this is yet to be implemented in most of these countries due to several factors including lack of political will, poor funding and in some cases poor uptake due to local cultural beliefs.

In 2008, the Vaccine Alliance (Gavi) made TCVs a priority as part of the typhoid investment initiative but did not make any financial commitments due to unavailability of a suitable vaccine [29], however, with the development of promising vaccine candidates with clinically appreciable efficacy albeit in the short- to-medium term, in November 2017, Gavi committed an $85 million funding window to support the roll out of these vaccines in eligible countries between 2019 and 2020 [7, 29] and in In January 2018, the WHO prequalified the TYPBAR-TCV. Since then, three countries have applied for support from Gavi, which includes a request for TCV use in response to an outbreak. The first Gavi-supported introduction of the TCV began in 2019 with Pakistan being the first country to request the vaccine in response to widespread transmission of an of extreme drug resistant strain (XDR) of *Salmonella typhi*. Following on this in the same year, Zimbabwe also applied for the TCV to combat an outbreak of drug resistant strains in Harare and was the first non-research use of the TCV in sub-Saharan Africa [29]. It is hoped that many more countries will apply for vaccine support and increase coverage to enhance reduction in global disease burden.

Another important factor driving an increase in disease burden is antibiotics resistance. This is mainly in endemic countries where the implementation of antibiotic stewardship is still a huge challenge. In these areas, a lot of antibiotics can be bought over-the-counter and patients often get them from the local chemist or patent medicine store without having to see a clinician. This has fostered the propagation of drug resistant strains of *Salmonella typhi* and *paratyphi* resulting in treatment failure, increased morbidity and mortality and increased tendency for chronic carriage. To mitigate this, there has to be a deliberate policy in these countries to better control access to antimicrobials, improved access to rapid diagnostic tests and public sanitation measures like clean, safe water, running pipe-borne water, clean toilet and waste disposal facilities.

8. Conclusion and recommendation

In conclusion, enteric fever caused by *Salmonella typhi* and *paratyphi* is still a huge global challenge and remains of major public health concern especially in low resource settings. The fundamental reasons for this unrelenting disease burden are multifactorial. Major factors include poor hygiene and sanitation measures, lack of access to portable drinking water, antibiotics resistance and poor access to vaccines. There has to be a strong political will for disease surveillance and primary care interventions such as ensuring the typhoid vaccines are included in routine immunisation schedules in endemic countries especially for children. The traditional typhoid vaccines held some challenges including convenience of administration and poor immunogenicity. However, the TCVs with better administration convenience especially in children as young as six months and longer-term protection, hold huge promise for the future of typhoid vaccine prophylaxis. Also, more countries need

to seize the opportunity for vaccine support provided by Gavi to improve vaccine uptake. There also has to be increased investment in research and development into novel vaccines and diagnostic tools which are accessible, available, reliable and affordable. With the aforementioned and improved commitment to environmental, food and hygiene status, we will hopefully combat this scourge and forge a better, healthier future with enteric fever disease burden reduced to the barest minimum.

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References

[1] Kirchhelle C, Pollard AJ, Vanderslott S. Typhoid-From Past to Future. Clin Infect Dis [Internet]. 2019;69(Suppl 5):S375-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31612936

[2] Chau TT, Campbell JI, Galindo CM, Van Minh Hoang N, Diep TS, Nga TTT, et al. Antimicrobial drug resistance of *Salmonella enterica* serovar typhi in asia and molecular mechanism of reduced susceptibility to the fluoroquinolones. Antimicrob Agents Chemother [Internet]. 2007 Dec;51(12):4315-23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17908946

[3] Kumar P, Kumar R. Enteric Fever. Indian J Pediatr [Internet]. 2017 Mar;84(3):227-30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27796818

[4] Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ [Internet]. 2004 May;82(5):346-53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15298225

[5] Crump JA, Ram PK, Gupta SK, Miller MA, Mintz ED. Part I. Analysis of data gaps pertaining to *Salmonella enterica* serotype Typhi infections in low and medium human development index countries, 1984-2005. Epidemiol Infect [Internet]. 2008 Apr;136(4):436-48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17686194

[6] Breiman RF, Cosmas L, Njuguna H, Audi A, Olack B, Ochieng JB, et al. Population-Based Incidence of Typhoid Fever in an Urban Informal Settlement and a Rural Area in Kenya: Implications for Typhoid Vaccine Use in Africa. Gosling RD, editor. PLoS One [Internet]. 2012 Jan 19;7(1):e29119. Available from: https://dx.plos.org/10.1371/journal.pone.0029119

[7] Gibani MM, Britto C, Pollard AJ. Typhoid and paratyphoid fever. Curr Opin Infect Dis [Internet]. 2018 Oct;31(5):440-8. Available from: http://journals.lww.com/00001432-201810000-00010

[8] Radhakrishnan A, Als D, Mintz ED, Crump JA, Stanaway J, Breiman RF, et al. Introductory Article on Global Burden and Epidemiology of Typhoid Fever. Am J Trop Med Hyg [Internet]. 2018 Sep 6;99(3_Suppl):4-9. Available from: http://www.ajtmh.org/content/journals/10.4269/ajtmh.18-0032

[9] Atamanalp SS, Aydinli B, Ozturk G, Oren D, Basoglu M, Yildirgan MI. Typhoid Intestinal Perforations: Twenty-six Year Experience. World J Surg [Internet]. 2007 Aug 20;31(9):1883-8. Available from: http://link.springer.com/10.1007/s00268-007-9141-0

[10] Sharma A, Sharma R, Sharma S, Sharma A, Soni D. Typhoid intestinal perforation: 24 Perforations in one patient. Ann Med Health Sci Res [Internet]. 2013;3(5):41. Available from: http://www.amhsr.org/text.asp?2013/3/5/41/121220

[11] Public Health England. NOIDs Weekly Report. Statutory Notifications of INfectious Diseases in England and Wales. Week 2020/53 Week ending 03/01/2021 [Internet]. 2021. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/949867/NOIDS-weekly-report-week53-2020.pdf

[12] Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. Clin Microbiol Rev [Internet]. 2015 Oct

15;28(4):901-37. Available from: https://cmr.asm.org/content/28/4/901

[13] Hart PJ, O'Shaughnessy CM, Siggins MK, Bobat S, Kingsley RA, Goulding DA, et al. Differential Killing of *Salmonella enterica* Serovar Typhi by Antibodies Targeting Vi and Lipopolysaccharide O:9 Antigen. Skurnik M, editor. PLoS One [Internet]. 2016 Jan 7;11(1):e0145945. Available from: https://dx.plos.org/10.1371/journal.pone.0145945

[14] Raffatellu M, Chessa D, Wilson RP, Dusold R, Rubino S, Bäumler AJ. The Vi Capsular Antigen of *Salmonella enterica* Serotype Typhi Reduces Toll-Like Receptor-Dependent Interleukin-8 Expression in the Intestinal Mucosa. Infect Immun [Internet]. 2005 Jun;73(6):3367-74. Available from: https://iai.asm.org/content/73/6/3367

[15] Raffatellu M, Chessa D, Wilson RP, Tükel C, Akçelik M, Bäumler AJ. Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. Infect Immun [Internet]. 2006 Jan;74(1):19-27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16368953

[16] Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med [Internet]. 2002 Nov 28;347(22):1770-82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12456854

[17] Crump JA. Progress in Typhoid Fever Epidemiology. Clin Infect Dis [Internet]. 2019 Feb 15;68(Supplement_1):S4-9. Available from: https://academic.oup.com/cid/article/68/Supplement_1/S4/5320155

[18] PHE. Recommendations for the Public Health Management of Gastrointestinal Infections. 2019; Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/614875/Public_Health_Operational_Guidelines_for_Typhoid_and_Paratyphoid.pdf

[19] Storey HL, Huang Y, Crudder C, Golden A, de los Santos T, Hawkins K. A Meta-Analysis of Typhoid Diagnostic Accuracy Studies: A Recommendation to Adopt a Standardized Composite Reference. Schallig HDFH, editor. PLoS One [Internet]. 2015 Nov 13;10(11):e0142364. Available from: https://dx.plos.org/10.1371/journal.pone.0142364

[20] Wijedoru L, Donegan S, Parry C. Rapid Diagnostic Tests for Typhoid and Paratyphoid (Enteric) Fever. In: Wijedoru L, editor. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2010. Available from: http://doi.wiley.com/10.1002/14651858.CD008892

[21] Olopoenia LA. Classic methods revisited: Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med J [Internet]. 2000 Feb 1;76(892):80-4. Available from: https://pmj.bmj.com/lookup/doi/10.1136/pmj.76.892.80

[22] Islam K, Sayeed MA, Hossen E, Khanam F, Charles RC, Andrews J, et al. Comparison of the Performance of the TPTest, Tubex, Typhidot and Widal Immunodiagnostic Assays and Blood Cultures in Detecting Patients with Typhoid Fever in Bangladesh, Including Using a Bayesian Latent Class Modeling Approach. Baker S, editor. PLoS Negl Trop Dis [Internet]. 2016 Apr 8;10(4):e0004558. Available from: https://dx.plos.org/10.1371/journal.pntd.0004558

[23] Kuhns M, Zautner AE, Rabsch W, Zimmermann O, Weig M, Bader O, et al. Rapid Discrimination of *Salmonella enterica* Serovar Typhi from Other Serovars by MALDI-TOF Mass Spectrometry. Chakravortty D,

editor. PLoS One [Internet]. 2012 Jun 29;7(6):e40004. Available from: https://dx.plos.org/10.1371/journal.pone.0040004

[24] Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid Fever. N Engl J Med [Internet]. 2002 Nov 28;347(22):1770-82. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra020201

[25] Zellweger RM, Basnyat B, Shrestha P, Prajapati KG, Dongol S, Sharma PK, et al. A 23-year retrospective investigation of Salmonella Typhi and Salmonella Paratyphi isolated in a tertiary Kathmandu hospital. PLoS Negl Trop Dis [Internet]. 2017 Nov;11(11):e0006051. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29176850

[26] Lin FYC, Ho VA, Khiem HB, Trach DD, Bay P Van, Thanh TC, et al. The Efficacy of a Salmonella typhi Vi Conjugate Vaccine in Two-to-Five-Year-Old Children. N Engl J Med [Internet]. 2001 Apr 26;344(17):1263-9. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200104263441701

[27] Szu SC. Development of Vi conjugate – a new generation of typhoid vaccine. Expert Rev Vaccines [Internet]. 2013 Nov 9;12(11):1273-86. Available from: http://www.tandfonline.com/doi/full/10.1586/14760584.2013.845529

[28] NHS England. Typhoid Vaccination [Internet]. Available from: https://www.nhs.uk/conditions/typhoid-fever/vaccination/

[29] Gavi. Typhoid Vaccine [Internet]. Available from: https://www.gavi.org/types-support/vaccine-support/typhoid