Typhoid Fever and Water

6

Varsha Gupta and Ritu Garg

Abstract

Salmonella enterica serotype Typhi and *Salmonella enterica* serotype Paratyphi A are the main causes of enteric fever in India, with *S.* Typhi being predominant. The principal habitat of Salmonella is the intestinal tract of humans and animals. Humans infected with salmonellae can carry the bacteria in the gut without signs of disease. Typhoid fever has been virtually eliminated from the advanced countries during the last several decades, mainly as a result of improvement in water supply and sanitation, but it continues to be endemic in the poor nations of the world. The control of paratyphoid fever has not been so successful. Infants, children, and adolescents in south-central and south-eastern Asia experience the greatest burden of illness. Studying interactions between the infected host and Typhi would improve our understanding of typhoid fever. Data on human typhoid are still scarce since Typhi uniquely infects humans, and there are no suitable animal models available. The hallmark of typhoid fever is prolonged, persistent fever. The definitive diagnosis still depends on isolation of the bacilli from the patient by blood culture . The prevalence of resistance to multiple first-line oral drugs has been rising. The wide distribution and high prevalence of MDR among *Salmonella* has led to fluoroquinolones assuming a primary role in the therapy for invasive salmonellosis. However, their widespread use has also been associated with decreased susceptibility and documented resistance to this class of drugs. Despite the availability and WHO's recommendation for the use of vaccines among school children in endemic areas, the use is quite limited. Typhoid fever thus continues to remain one of the important water and food borne diseases.

Keywords

Salmonella Typhi · Fluoroquinolone · Diagnosis

V. Gupta (✉) · R. Garg
Department of Microbiology, Govt. Medical College and Hospital, Chandigarh 160031, India
e-mail: varshagupta_99@yahoo.com

P. P. Singh and V. Sharma (eds.), *Water and Health*,
DOI: 10.1007/978-81-322-1029-0_6,

Introduction

The genus *Salmonella* was designated by Lignières in 1900 (Le Minor 2003; Popoff and Le Minor 2005). The genus *Salmonella*, a member of the family *Enterobacteriaceae*, includes Gram-negative motile straight rods. *S. enterica* subsp. *enterica* serovar Enteritidis is the most frequently isolated serovar from humans all over the world. Salmonellae pathogenic to humans can cause two types of disease: typhoid and paratyphoid fever and gastroenteritis (Le Minor 2003; Fenwick 2006).

Enteric fever is a disease of major public health problem in our country. *Salmonella enterica* serotype Typhi and *S. enterica* serotype Paratyphi A are the main causes of enteric fever in India, with *Salmonella* Typhi being predominant (Typhoid fever) (House et al. 2001). Salmonella usually causes an intestinal infection that often lasts 1 week or longer. The principal habitat of *Salmonella* is the intestinal tract of humans and animals (Le Minor 2003). Salmonellae are constantly found in environmental samples, because they are excreted by humans, pets, farm animals, and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters (Popoff and Le Minor 2005; Arvanitidou et al. 2005). Salmonellae do not seem to multiply significantly in the natural environment, but they can survive several weeks in water and in soil if conditions of temperature, humidity, and pH are favorable (Le Minor 2003).

Salmonellae isolated from environmental sources are predominantly non-Typhi or Paratyphi serovars. The great majority of the strains are isolated from poultry, red meat, milk and dairy products, vegetables, and fruits.

Unlike cholera, humans infected with salmonellae can carry the bacteria in the gut without signs of disease. Infected humans can harbor the bacteria for considerable periods of time. About 5 % of patients clinically cured from typhoid fever remain carriers for months or even years. These people can be chronic holders of the bacterium in the gut and constitute the main reservoir of the bacteria in the environment (Popoff and Le Minor 2005).

The salmonellosis cycle in the environment can involve shellfish also. Salmonellae survive sewage treatments if suitable germicides are not used in sewage processing. If effluent from the sewage plant passes into a coastal area, edible shellfish (mussels, oysters) can become contaminated. Shellfish concentrate bacteria as they filter several liters of water per hour. Ingestion by humans of these seafoods (uncooked or superficially cooked) may cause typhoid fever or other salmonellosis (Popoff and Le Minor 2005).

Thus, human illness is linked with foods of animal origin. It is also transmitted by direct contact with animals, non-animal foods, by water and occasionally by human contact. So, typhoid fever is one of the important waterborne and foodborne diseases (Swaminathan et al. 2005). It has been observed that the incidence of typhoid fever decreases when the level of development of a country increases (i.e., controlled water sewage systems, pasteurization of milk and dairy products). Where these hygienic conditions are missing, the probability of fecal contamination of water and food remains high and so is the incidence of typhoid fever (Cabral 2010).

Typhoid fever is a distinctive acute systemic febrile infection of the mononuclear phagocytes. *S. enterica* subsp. *enterica* serotype Typhi is the etiological agent of typhoid fever (House et al. 2001). The name typhoid was given by Louis in 1829, as a derivative from typhus (Udeze et al. 2010). In India, though, *S. enterica* serotype Typhi remains the predominant *Salmonella* species causing enteric fever but it may be caused by several serovars like *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Paratyphi B, and occasionally *Salmonella typhimurium* (Bhattacharya et al. 2011). Isolation of *Salmonella* serotype Paratyphi A causing the same disease (paratyphoid fever) has also been reported increasingly (Chandel et al 2000; Sood et al. 1999). Salmonellae currently comprise above 2000 serotypes or species, all of them potentially pathogenic. Infections in humans include typhoid fever, focal systemic infections, septicemia, and

most commonly diarrhea, varying from acute watery diarrhea to bloody diarrhea or dysentery. Salmonellae are ecologically entrepreneurial and exist in a multiplicity of habits; this characteristic adaptability, which accounts for the ubiquity of the organisms in nature and for the many ways in which they encounter potential new human hosts, is related to their genetic plasticity (Keusch GT 1998). The risk factors such as poor sanitation, lack of a safe drinking water supply, and low socioeconomic conditions in resource-poor countries are amplified by the evolution of multidrug-resistant *salmonellae* which is associated with increased mortality and morbidity associated with typhoid fever (Kanungo et al. 2008).

Morphology

The organism is a short bacillus about 2–4× 0.6 μm in size. It is motile due to its peritrichous flagella (Arora 2008).

Culture Characteristics

Salmonella Typhi is an aerobic and facultative anaerobic bacteria. It grows readily on simple media. It can grow at pH 6–8 and temperature requirement is 15–41 °C (optimum 37 °C). On MacConkey and deoxycholate citrate media, colonies are colorless due to the absence of lactose fermentation. On Wilson and Blair bismuth sulfite medium, jet black colonies with a metallic sheen are formed due to production of H_2S (Ananthanarayan and Jayaram Paniker 2005).

Biochemical Reactions

Salmonella Typhi ferments glucose, mannitol, and maltose, forming acid without gas. Lactose, sucrose, and salicin are not fermented. Indole is not reduced. They are MR positive, VP negative, and citrate negative. Urea is not hydrolyzed. H_2S is produced (Ananthanarayan and Jayaram Paniker 2005).

Sources of Infection

The sources of infection are food and drink that have been contaminated with salmonellae. The following sources are important:

1. Water—Contamination with feces often results in explosive epidemics.
2. Milk and other dairy products (ice cream, cheese, custard)—Contamination with feces and inadequate pasteurization or improper handling. Some outbreaks are traceable to the source of supply.
3. Shellfish—From contaminated water.
4. Dried or frozen eggs—From infected fowl or contaminated during processing.
5. Meats and meat products—From infected animals (poultry) or contamination with feces by rodents or humans.
6. Recreational drugs—Marijuana and other drugs.
7. Animal dyes—Dyes (carmine) used in drugs, foods, and cosmetics.
8. Household pets—Turtles, dogs, cats, etc. (Brooks et al. 2004).

Transmission

S. Typhi has no non-human vectors. The following are modes of transmission:

- Oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine.
- Hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene.
- Oral transmission via sewage-contaminated water or shellfish (especially in the developing world (Earampamoorthy and Koff 1975).

An inoculum as small as 100,000 organisms causes infection in more than 50 % of healthy volunteers (Levine et al. 2001).

Epidemiology

Worldwide, 16 million cases are estimated to occur annually with 600,000 deaths. The proportion of typhoid to paratyphoid A is about 10:1. Paratyphoid B is rare and C very rare. In the year 2000, it was estimated that typhoid fever caused 21.7 million illnesses and 217,000 deaths and paratyphoid fever 5.4 million illnesses worldwide. Infants, children, and adolescents in south-central and south-eastern Asia experience the greatest burden of illness (Crump et al. 2004). The age incidence is related to the endemicity of the disease and the level of sanitation.

Typhoid fever has been virtually eliminated from the advanced countries during the last several decades mainly as a result of improvement in water supply and sanitation but it continues to be endemic in the poor nations of the world. The control of paratyphoid fever has not been so successful. The distribution of paratyphoid bacilli shows marked geographical differences. *S.* Paratyphi A is prevalent in India and other Asian countries, Eastern Europe, and South America; *Salmonella* Paratyphi B in Western Europe, Britain, and North America; and *Salmonella* Paratyphi C in Eastern Europe and Guyana.

Epidemiologic Trends

Despite the limitations of currently available epidemiologic data, a number of recent trends in enteric disease epidemiology have emerged in the African, Asian, and Latin American regions. In sub-Saharan Africa where the burden of enteric fever is the least well characterized, hospital-based studies indicate that non-Typhi serotypes of *Salmonella*, particularly *Salmonella enteritidis* and *Salmonella*. *typhimurium*, greatly outnumber *Salmonella* Typhi and *Salmonella* Paratyphi as causes of bloodstream infection (Shaw et al. 2008; Mweu and English 2008). In Asia, a large population-based prospective study using standardized surveillance methods has estimated typhoid fever incidence in China, India, Indonesia, Pakistan, and Vietnam. This study confirmed the high incidence of typhoid fever in the region, particularly among children and adolescents, but also demonstrated that substantial variation in incidence occurs between surveillance sites in the same region (Ochiai et al. 2008). Simultaneously, *Salmonella* Paratyphi A appears to be responsible for a growing proportion of enteric fever in a number of Asian countries, sometimes accounting for 50 % of *Salmonella* bloodstream isolates among enteric fever patients.

Enteric fever is endemic in all parts of India. There have been two large-scale studies in India on the incidence of blood culture-confirmed typhoid fever, one among individuals under 40 years old (Sinha et al. 1999) and another among children 6–17 years old (Chuttani et al. 1977), but as yet, none on paratyphoid fever. Thus, the actual burden of paratyphoid fever in India and its incidence and characteristics relative to typhoid fever are poorly understood. In a study conducted in Punjab that examined 340 enteric fever cases, 334 *Salmonella* Typhi and 6 *Salmonella* Paratyphi A isolates were identified (Pathania and Sachar 1965). This scenario, however, has changed as recent studies have highlighted the increasing occurrence of paratyphoid fever (Rodrigues et al. 2003).

A large-scale community study performed in an Indian urban slum showed incidence as high as 2 per 1,000 population per year for children under five, and 5.1 per 1,000 population per year for children under ten (Sur et al. 2006). Another study in Northern India showed that the majority of cases occurred in children aged 5–12 years and 24.8 % of cases were in children up to 5 years of age (Walia et al. 2006). *Salmonella* serovars showed an age-related bias, with paratyphoid fever more common in adults. One study from Kolkata showed the incidence of paratyphoid fever was lower (0.8/1000/year), and the mean age of paratyphoid patients was older

(17.1 years) compared to typhoid fever (incidence 1.4/1000/year, mean age 14.7 years) (Sur et al. 2007; Kanungo et al. 2008).

The source of infection is a patient or, far more frequently, a carrier. Patients who continue to shed typhoid bacilli in feces for 3 weeks–3 months after clinical cure are called convalescent carriers. Those who shed the bacilli for more than three months but less than a year are called temporary carriers and those who shed the bacilli for over a year are called chronic carriers. About 2–4 % of patients become chronic carriers. The development of the carrier state is more common in women and in the older age groups. Some persons may become carriers following inapparent infections (symptomless excretor). The shedding of bacilli is usually intermittent. The bacilli persist in the gall bladder or kidney and are eliminated in the feces or urine. Urinary carriage is less frequent.

The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, for example, when carriers working as food handlers are shedding organisms. The best known of such typhoid carriers was Mary Mallon (Typhoid Mary), a New York cook who, over a period of 15 years, caused at least seven outbreaks affecting over 200 persons.

Typhoid fever occurs in two epidemiological types. The first is endemic or residual typhoid that occurs throughout the year though seasonal variations may sometimes be apparent. Some studies show a peak of the disease from July to September, as it coincides with the rainy season when the chance of water contamination is high, especially in crowded areas (Sur et al. 2006). The second is epidemic typhoid, which may occur in endemic or non-endemic areas. Typhoid epidemics are usually water, milk, or foodborne (Ananthanarayan and Jayaram Paniker 2005).

Pathogenicity

Infection is initiated by oral ingestion of organisms, which must pass the gastric acid barrier to establish infection. Salmonella exhibits a genetic adaptive acid tolerance response. Exposure to acid leads to synthesis of at least 40 proteins, some of which may play a role in pathogenesis. Bacteria successfully evading "acid death" in the stomach pass on to the distal ileum and colon, where they penetrate the mucosal barrier.

Initial bacterial invasion results in transient asymptomatic bacteraemia, as organisms are rapidly ingested by mononuclear phagocytes within which they survive and multiply. This process is favored by a lack of bactericidal antibodies in the susceptible host; in contrast, opsonized salmonellae are taken up by neutrophils and killed. Opsonophagocytosis is limited by the capsular Vi polysaccharides of *Salmonella* Typhi which enhances resistance to complement activation and bacterial lysis by the alternative pathway as well as to peroxide-mediated killing. The fate of organisms within macrophages depends both on microbial factors that promote resistance to killing and on specific host T lymphocyte-activated, cell-mediated immune mechanisms that are under the genetic control of the pathogenicity locus in mice. *S. enterica* serovar Typhi uses SPI-1 TSS3 to invade the intestinal epithelium and SPI-2 TSS3 to survive in macrophages and employs additional virulence factors to overcome the mucosal barrier and to colonize internal organs (Casadesús 2011). When intracellular multiplication has proceeded enough to permit the initiation of persistent bacteremia, the clinical phase of typhoid fever begins with invasion of the gallbladder and Peyer's patches of the intestine (Parry et al. 2002). The sustained bacteremia is responsible for the persistent fever of clinical typhoid, while inflammatory responses to tissue

invasion determine the pattern of clinical expression (cholecystitis, intestinal hemorrhage, or perforation).With invasion of the gallbladder and Peyer's patches, bacteria regain entry to the bowel lumen and may be recovered in stool cultures beginning in the second week of clinical disease. Seeding of the kidney leads to positive urine cultures. The lipopolysaccharide of *Salmonella* Typhi may contribute to fever, leucopenia, and other systemic symptoms, but the occurrence of such symptoms in individuals rendered tolerant to endotoxin supports a role for other factors, such as cytokines released from infected mononuclear phagocytes, that can mediate inflammation (Keusch GT 1998).

Studying interactions between the infected host and Typhi would improve our understanding of typhoid fever. Typhi has developed remarkable persistence mechanisms within the host that help ensure its survival and transmission. However, data on human typhoid collected by using modern immunological and molecular techniques are scarce since Typhi uniquely infects humans, and there are no suitable animal models available. As survival within macrophages is an essential step for *Salmonella* pathogenesis, macrophages represent a useful model to study Typhi. Elucidating the bacterial genes expressed in the host and those underlying typhoid pathogenesis should lead to the development of new strategies including novel anti-bacterial treatments and identification of novel vaccine candidates to control the disease (Daigle 2008).

Clinical Manifestations

The incubation period of typhoid fever is variable, that is, 3–60 days and depends on both the inoculum size and the state of the host's defense. The disease classically presents with a step like daily increase in temperature associated with headache, malaise, and chills. The hallmark of typhoid fever is prolonged, persistent fever (4–8 weeks in untreated patients). The illness may also be mild and brief. In some cases, acute, severe infection with disseminated intravascular coagulation and central nervous system involvement rapidly results in death. In other instances, necrotizing cholecystitis or intestinal bleeding and perforation can occur in the third or fourth week of illness, when the patient is otherwise improving. In most cases, the onset of these complications is dramatic and clinically obvious. Intestinal perforation appears to be less common among children under 5 years of age.

Early intestinal manifestations include constipation (especially in adults) or mild diarrhea (in children) associated with abdominal tenderness. Mild hepatomegaly is detectable in the majority of patients. Bradycardia relative to the height of the fever may be clinical clue to typhoid but is present in only a minority of patients. Epistaxis may be noted in the early stages of illness. "Rose spots" appearing as small, pale red, blanching, slightly raised macules are occasionally seen on the chest and abdomen during the first week. They can evolve into non-blanching small hemorrhages that are difficult to see in dark skinned patients. The major characteristics of untreated typhoid are persistent high fever, severe anorexia, weight loss, and changes in sensorium, but a variety of other complications also may develop including hepatitis, meningitis, nephritis, myocarditis, bronchitis, pneumonia, arthritis, osteomyelitis, parotitis, and orchitis. Except for that or relapse, the frequency of all these complications, including hemorrhage and perforation, is reduced by prompt use of appropriate antibiotics. Typhoid, long considered to be uncommon and typically mild in young children, may in reality be frequent and severe in this group. Neonatal typhoid, which can be acquired vertically from infected mother or from exogenous sources, is often a severe, life-threatening septicemic illness with a high case fatality rate.

As multidrug-resistant *Salmonella* Typhi is becoming more prevalent in many endemic countries, the patients infected with resistant strains present with more severe illness, look toxic, and have a higher incidence of disseminated intravascular coagulation and hepatomegaly and a threefold higher mortality rate that is thought to be related to the longer duration of disease and to prior ineffective oral antibiotic therapy.

Around 3–5 % of patients become long-term asymptomatic carriers, some for life unless treated. Many carriers give no history of typhoid fever and probably have an undiagnosed mild infection (Keusch GT 1998).

Mortality/Morbidity

With prompt and appropriate antibiotic therapy, typhoid fever is typically a short-term febrile illness requiring a median of 6 days of hospitalization. When treated, it has few long-term sequelae and a 0.2 % risk of mortality (Lynch et al. 2009). Untreated typhoid fever is a life-threatening illness of several weeks' duration with long-term morbidity often involving the central nervous system. The case fatality rate in the United States in the pre-antibiotic era was 9–13 % (Crump et al. 2008).

Immunity

Infections with *Salmonella* Typhi or *Salmonella* Paratyphi usually confer a certain degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease. However, relapse may occur in 2–3 weeks after recovery in spite of antibodies, secretory IgA antibodies may prevent attachment of salmonellae to intestinal epithelium.

Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to salmonella infections, particularly osteomyelitis. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (Brooks et al. 2004).

Laboratory Diagnosis

In around 25 % of patients, leucopenia and neutropenia are evident. In most patients, the white blood cell count is normal or low in relation to the degree of fever; this pattern may be a clue to the diagnosis. Whereas definitive diagnosis still depends on isolation of the bacilli from the patient, yield of culture is disappointingly low. The yield is affected by the overall several variables: when the cultures are performed, what is cultured, and whether the patient has taken antibiotics. Other tests for diagnosis are the demonstration of antibodies in the patient serum. A positive blood culture is diagnostic. Demonstration of antibodies is not conclusive evidence of current infection. A third method is the demonstration of typhoid bacillus antigen in blood or urine (Keusch GT 1998).

Blood culture: Bacteremia occurs early in the disease and blood cultures are positive in approximately 90 % of cases in the first week of fever. The popular belief that blood culture for diagnosis of typhoid fever is useful only in the first week is not true. Blood culture is positive in approximately 75 % of cases in the second week, 60 % in the third week, and 25 % thereafter till the subsidence of pyrexia. Blood culture rapidly becomes negative on treatment with antibiotics.

About 5–10 ml of blood is collected by venipuncture after proper preparation of site and inoculated into a culture bottle containing 50–100 ml of 0.5 % bile broth. Blood contains substances that inhibit the growth of the bacilli and hence it is essential that the broth be taken in sufficient quantity to provide at least fourfold dilution of blood.

After incubation overnight at 37 °C, the bile broth is subcultured on MacConkey agar. Pale non-lactose fermenting colonies that may appear on this medium should be identified. Confirm the isolate by further putting up biochemical reactions and performing slide agglutination. For identification of unusual serotypes, the help of the National Salmonella Reference Centre in India is located at the Central Research Institute, Kasauli. The reference centre for salmonellae of animal origin is at the Indian Veterinary Research Institute, Izatnagar.

If salmonellae are not obtained from the first subculture from bile broth, subcultures should be repeated every other day till growth is obtained. Cultures should be declared negative only after incubation for 10 days. An alternative to blood culture is the clot culture. Clot cultures yield a

higher rate of isolation than blood cultures as the bactericidal action of the serum is obviated. Another advantage is that a sample of serum also becomes available. Even though agglutinins may be absent in the early stages of the disease, a Widal test provides a baseline titer against which the results of tests performed later may be evaluated.

Feces culture: Salmonellae are shed in feces throughout the course of the disease and even in convalescence, with varying frequency. Hence, fecal cultures are almost as valuable as blood cultures in diagnosis. A positive fecal culture, however, may occur in carriers as well as in patients. The use of enrichment and selective media and repeated sampling increase the rate of isolation. Fecal culture is particularly valuable in patients on antibiotics as the drug does not eliminate the bacilli from the gut as rapidly as it does from the blood.

Fecal samples are plated directly on MacConkey, DCA and Wilson-Blair media. For enrichment, specimens are inoculated into one tube each of selenite and tetrathionate broth and incubated for 12–18 h before subculture onto plates.

Urine culture: Salmonellae are shed in the urine irregularly and infrequently. Hence, urine culture is less useful than the culture of blood or feces. Cultures are generally positive only in the second and third weeks and then only in about 25 % of cases. Repeat sampling improves the rate of isolation.

Other materials for culture: Bone marrow culture is valuable as it is positive in most cases even when blood cultures are negative. Culture of bile obtained by duodenal aspiration is usually positive and may be employed for the detection of carriers. Other materials which may yield isolation at times are rose spots, pus from suppurative lesions, CSF, and sputum (Ananthanarayan and Jayaram Paniker 2005).

Serologic Methods

Widal test (Tube dilution agglutination test): Serum agglutinins rise sharply during the second and third weeks of salmonella infection. At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer. Serial (twofold) dilutions of unknown serum are tested against antigens from representative salmonellae. The results are interpreted as follows: (1) High or rising titer of O (>1:160) suggests that active infection is present. (2) High titer of H (>1:160) suggests past immunization or past infection. (3) High titer of antibody to the Vi antigen occurs in some carriers. Results of serologic tests for salmonella infection must be interpreted cautiously. The possible presence of cross-reactive antibodies limits the use of serology in the diagnosis of salmonella infections (Brooks et al. 2004). According to studies in developing countries with high incidence of typhoid fever, coagglutination is much more reliable than culture because so many patients have already taken antibiotics before being seen by a physician. New enzyme immunoassays that detect *S.* Typhi outer membrane proteins are being evaluated. A sensitive and specific multiplex polymerase chain reaction for Vi antigen promises to be highly sensitive and specific, but its value has not been proved in clinical studies, nor is it available yet (Keusch GT 1998).

Detection of carriers: The detection of carriers is important for epidemiological and public health purposes. Laboratory tests are also useful in screening food handlers and cooks to detect carrier state.

The identification of fecal carriers is by isolation of the bacillus from feces or from bile. The frequency and intensity of bacillary shedding vary widely, and it is essential to test repeated samples. Cholagogue purgatives increase the chance of isolation. For the detection of urinary carriers, repeated urine culture should be carried out.

The Widal reaction is of no value in the detection of carriers in endemic countries. The demonstration of Vi agglutinins has been claimed to indicate the carrier state. While this is useful as a screening test, confirmation should be made by culture.

The tracing of carriers in cities may be accomplished by the “sewer-swab” technique. Gauze pads left in sewers and drains are

cultured, and by tracing positive swabs, one may be led to the house harboring a carrier. Another technique of isolating salmonellae from sewage is filtration through Millipore membranes and culturing the membranes on highly selective media such as Wilson and Blair media.

Bacteriophage Typing

Intraspecies classification of *S.* Typhi for epidemiological purposes was made possible by bacteriophage typing, first developed by Craigie and Yen in 1937. Apart from helping in tracing the source of epidemics, phage typing also provides information on the trends and pattern in the epidemiology of typhoid at the local, national, and international levels. Phage typing is carried out at the National Phage Typing Centre and is coordinated by the International Reference Centre. The National Salmonella Phage Typing Centre for India is located at the Lady Hardinge Medical College, New Delhi. Phage types A and F1 are the most common and are present throughout India (Ananthanarayan and Jayaram Paniker 2005).

Differential Diagnosis

When all the classic clinical manifestations are present, including rose spots, prolonged fever, relative bradycardia, and leucopenia, the diagnosis of typhoid will be strongly suggested. However, most cases do not fit this "typical" profile. Differential diagnosis includes infections associated with prolonged fevers, such as the rickettsioses, brucellosis, tularemia, leptospirosis, military tuberculosis, viral hepatitis, infectious mononucleosis, CMV, and malaria, as well as non-infectious causes of fever, such as lymphomas (Keusch GT 1998).

Treatment

Specific antibacterial therapy for enteric fever became available only in 1948 with the introduction of chloramphenicol, which continued as gold standard antimicrobial till the 1970s, after that resistance became common to this drug. No drug has been better in promoting a favorable clinical response, which usually becomes apparent within 24–48 h of the start of treatment in the appropriate dosages. Other effective oral regimens include amoxicillin, trimethoprim-sulfamethoxazole or for patients over 17 years of age, fluoroquinolones such as ciprofloxacin or ofloxacin.

A variety of intravenous drugs are also effective. Both chloramphenicol and trimethoprim-sulfamethoxazole can be given intravenously to patients who cannot take oral medications. Other effective parenteral antimicrobials include high-dose ampicillin, cefotaxime, cefoperazone, and 4-fluoroquinolones. However, none has been as rapidly acting or as effective as ceftriaxone. In addition, compared with that for other drugs, the relapse rate for ceftriaxone appears lower.

The prevalence of resistance to multiple first-line oral drugs has been rising among strains of *Salmonella* Typhi in developing countries, especially in the Indian subcontinent and Southeast Asia, due to the acquisition of plasmids encoding inactivating ß-lactamases and chloramphenicol acetyl transferases. Where multidrug resistance is a problem, ceftriaxone or a 4-fluoroquinolone should be administered initially to adults over 17 years of age, and ceftriaxone is the best choice for children because of concerns about quinolone induced arthropathy and cartilage damage in this age group. Short-course quinolone therapy has been shown to be effective against multidrug-resistant typhoid in children, and its use further reduces the

likelihood of drug toxicity. Alternative oral agents that reportedly are effective for this indication include furazolidine and cefixime. Recent clinical trials suggest that azithromycin 500 mg once daily for 7 days in adults or azithromycin 20 mg/kg/day up to a maximum of 1,000 mg/day for 7 days in children is useful for the management of uncomplicated typhoid fever (Effa and Bukirwa 2008).

Eradication of the chronic carrier state, especially in the presence of gallstones, is notoriously difficult. Traditional regimens have used ampicillin or amoxicillin plus probenecid or trimethoprim-sulfamethoxazole plus rifampicin for at least 6 weeks. Recent studies suggest that a 4 week course of a 4-fluoroquinolone is at least as good and probably much better because the organism is exquisitely sensitive in vitro and the drugs reach the gut lumen, liver, gall bladder, and bile in active form. The new quinolones provide the best chance of eradicating *S.* Typhi in the presence of gallstones (Keusch GT 1998).

Antimicrobial Resistance

Antimicrobial resistance is a major public health problem in both *Salmonella* Typhi and *Salmonella* Paratyphi, and timely treatment with appropriate antimicrobial agents is important for reducing the mortality of enteric fever (Edelman and Levine 1986).

Resistance to chloramphenicol did not pose any problem in typhoid fever till 1972, when resistant strains emerged in Mexico and in Kerala (India). In Mexico; the resistant strain caused an explosive epidemic, with high mortality. Travelers who got infected in Mexico had, on occasion, conveyed the resistant strain to North America and Europe but it did not get established in these areas. Chloramphenicol-resistant typhoid fever has become a problem in many countries in Asia.

In India, chloramphenicol-resistant typhoid fever appeared in epidemic form first in Calicut (Kerala) in early 1972. It became endemic and was confined to Kerala till 1978. Subsequently, such strains carrying drug resistance plasmids appeared in many other parts of India. Though resistant to chloramphenicol, such strains were initially sensitive to ampicillin, amoxicillin, cotrimoxazole, and furazolidone, which were successfully used for treatment. By late 1980s, typhoid bacillus strains resistant to many or all of these drugs began to spread in most parts of India. At present, the drugs useful in treatment of such multiresistant typhoid cases are the later fluoroquinolones (such as ciprofloxacin, pefloxacin, ofloxacin) and the third-generation cephalosporins (such as ceftazidime, ceftriaxone, cefotaxime). Furazolidone is still active against most isolates but its action is too slow for it to be used alone in treatment. Recently, many strains have become resistant to fluoroquinolones, but several isolates of typhoid bacilli are now sensitive to chloramphenicol (Ananthanarayan and Jayaram Paniker 2005; Gupta et al. 2009).

Multiple Drug Resistance

Resistance to the traditional first-line antimicrobial agents ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole defines multiple drug resistance (MDR) in *Salmonella*. The MDR phenotype has been shown to be widespread among *Salmonella* Typhi for many years (Rowe et al. 1997) and is present, albeit at lower rates, among *Salmonella* Paratyphi (Gupta et al. 2008; Parry and Threlfall 2008). Surveillance studies demonstrate considerable geographic variation in the proportion of *Salmonella* Typhi isolates that are MDR within the same region, with sites in India, Pakistan, and Vietnam having higher rates of MDR than sites in China and Indonesia (Ochiai et al. 2008). Furthermore, longitudinal studies at the same site demonstrate marked changes in the proportion of *Salmonella* Typhi and *Salmonella* Paratyphi A with MDR over time, including reductions in the proportion of isolates with MDR (Maskey et al. 2008).

The wide distribution and high prevalence of MDR among *Salmonella* have led to fluoroquinolones assuming a primary role in the therapy for invasive salmonellosis. Some investigators have noted increase in the

prevalence of more susceptible *Salmonella* Typhi and *Salmonella* Paratyphi strains coinciding with a switch from traditional first-line antimicrobials to fluoroquinolones for the management of enteric fever (Zaki and Karande 2011; Sood et al. 1999). However, the widespread use of fluoroquinolones has also been associated with decreased susceptibility (Lynch et al. 2009) and documented resistance to this class of drugs (Brown et al. 1996). A single chromosomal mutation in the quinolone resistance determining region (QRDR) of the *gyrA* gene may be sufficient to result in decreased ciprofloxacin susceptibility (DCS). Nalidixic acid resistance in the presence of ciprofloxacin susceptibility had been thought to be a reliable indicator of DCS, but this is now known not to be the case and many have suggested that DCS is most reliably determined by measurement of the ciprofloxacin minimum inhibitory concentration (MIC) (Crump et al. 2003; Threlfall et al. 1999). Patients with enteric fever due to isolates with DCS are more likely to have prolonged fever clearance times and higher rates of treatment failure (Crump et al. 2008). In the United States, MDR and DCS *Salmonella* Typhi are associated with travel to the Indian subcontinent (Lynch et al. 2009). In addition to DCS, ciprofloxacin resistance has been reported among both *Salmonella* Typhi (Chuang et al. 2009) and *Salmonella* Paratyphi A (Maskey et al. 2008).

As fluoroquinolone use continues to expand and as DCS and fluoroquinolone resistance drive the use of third-generation cephalosporins and other agents for the management of enteric fever, new patterns of antimicrobial resistance can be anticipated. Patterns of antimicrobial resistance seen in non-Typhi *Salmonella* and Enterobactericeae may emerge in *Salmonella* Typhi and *Salmonella* Paratyphi. Although quinolone resistance among Enterobactericeae usually arises due to mutations in the QRDR of *gyrA*, plasmid-mediated resistance is increasingly recognized. Plasmid-mediated quinolone resistance is associated with *qnr* genes that encode a protein that protects DNA gyrase from ciprofloxacin and by *aac(6′)-Ib-cr*, an aminoglycoside-modifying enzyme with activity against ciprofloxacin (Parry and Threlfall 2008). Plasmids bearing *qnr* or *aac(6′)-Ib-cr* may also contain an extended-spectrum cephalosporin resistance gene, which would pose a threat to the success of two major antimicrobial classes for the management of invasive salmonellosis. *Salmonella* Typhi isolate producing an SHV-12 extended-spectrum beta-lactamase (ESBL) (Al Naiemi et al. 2008) and ESBL-producing *Salmonella*Paratyphi A have recently been reported (Pokharel et al. 2006). Of further concern, rare non-Typhi *Salmonella* isolates have been described containing the carbapenemase, *bla*IMP-4 as well as *qnrB4* conferring both meropenem resistance and DCS (Nordmann et al. 2008).

Prevention and Control

Since the main route of typhoid transmission is feco-oral by contaminated food and water, the disease remains a serious problem in India where it is confounded by low socioeconomic conditions and overcrowding. Food and water safety are complex issues that depend on a number of interrelated environmental, cultural, and socioeconomic factors. Education of consumers and food handlers about basic principles of safe food handling is an important aspect of prevention but is insufficient by itself. The general strategy of prevention of foodborne and waterborne disease is to understand the mechanisms by which contamination and disease transmission occur and to institute appropriate prevention measures. As humans are the only reservoir of this feco-orally transmitted disease, preventive measures include improvement of water supply and sanitation facilities. Water treatment, waste disposal, and protecting the food supply from contamination are important public health measures. Carriers of typhoid must not be allowed to work as food handlers (Giannella 2010; Lima and Guerrant 2009). Where this approach is not yet possible and for travelers, immunization has been used.

Vaccines

The concept of vaccination against typhoid began in the 1960s when field trials showed the effectiveness of a killed vaccine, reporting a protection rate of approximately 70 % after two doses (Levine et al. 1999). Traditional heat killed, phenol preserved, whole-cell typhoid and paratyphoid vaccine constituting *Salmonella* Typhi, *Salmonella* Paratyphi A, and *Salmonella* Paratyphi B is no longer recommended because of its limited efficacy and duration of protection and the high frequency of local reactions and fever.

One option for children over 6 years of age and adults consists of three doses of a first generation the live, attenuated oral vaccine, Ty21a, which is invasive but metabolically defective and dies after a few cycles of replication. This vaccine is safe, provides as much protection as the killed vaccine, and continues to be protective for at least several years.

One dose of purified Vi polysaccharides vaccine has proved as effective and long lasting as multiple doses of Ty21a and may be used in children over 2 years of age and in at-risk HIV-infected patients.

Vi stimulates the IgG antibody, while Ty21a induces humoral and cell-mediated immune responses but not the Vi antibody (Levine 2001).

New genetically engineered live typhoid vaccine strains are being developed, not only for immunization against typhoid but also for use as live vectors into which extraneous genes can be cloned for oral delivery of protective antigens from unrelated species. In addition, Vi protein conjugates are being evaluated as immunogens suitable for infants, especially in endemic regions where infantile typhoid is prevalent and remains a dangerous disease (Keusch 1998). The immunologic properties of Vi polysaccharide (Vi) were improved by binding it to a recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA). Vi-rEPA was 89 % effective at preventing blood culture-confirmed typhoid fever in 2–5-year-olds and induced high levels of serum IgG anti-Vi. A minimal protective level of 3.5 enzyme-linked immunosorbent assay units [ELISA units (EU)] was inferred from the level of anti-Vi 46 months after immunization. The authors conclude that Vi-rEPA was safe, induced protective anti-Vi levels, and was compatible with EPI vaccines, and it can be used in infants (Thiem et al. 2011).

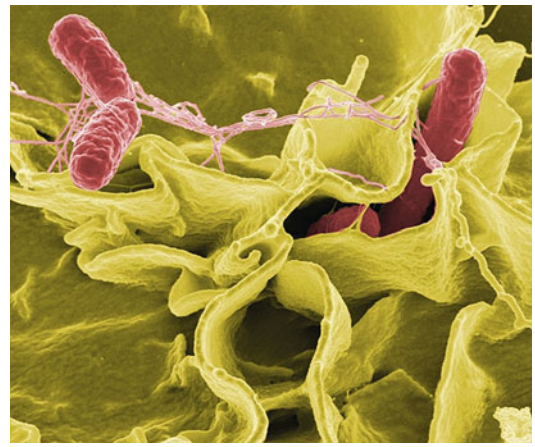

Fig. 6.1 Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (*red*) invading cultured human cells *Credit*: Rocky Mountain Laboratories, NIAID, NIH Source: [http://www2.niaid.nih.gov/biodefense/public/images.htm NIAID] This image is a work of the National Institutes of Health, part of the United States Department of Health and Human Services. As a work of the U.S. federal government, the image is in public domain.

Despite the availability of these vaccines and the WHO's recommendation for the use of vaccines among school children in endemic areas, the use is quite limited because of cost, lack of proper data, and the vaccine's ineffectiveness in children under 2 years of age. In view of the increasing number of infections with *Salmonella* Paratyphi A, development of a suitable vaccine against *Salmonella* Paratyphi A is urgently needed (Kanungo et al. 2008).

Conclusions

The existence of multidrug-resistant bacteria is a serious and growing problem in the treatment of typhoid, especially in the developing world. Isolation and characterization of MDRST from

all regions of the world for effective epidemiologic surveillance and control should continue with intensive scrutiny of *Salmonella* Typhi strains from developing countries. Drug resistance in bacteria results in increase in morbidity and mortality rates associated with the disease. So, in view of the re-emergence of sensitivity to first-line drugs, large-scale systematic studies are required to determine whether these drugs can again be used for the treatment of typhoid fever in developing countries in future times to come. Finally, acceleration of the conjugate vaccine programmes should be done for global adoption (Fig. 6.1).

References

Al Naiemi N, Zwart B, Rijnsburger MC, Roosendaal R, Debets-Ossenkopp YJ, Mulder JA et al (2008) Extended-spectrum-beta-lactamase production in a *Salmonella enterica* serotype Typhi strain from the Philippines. J Clin Microbiol 46:2794–2795

Ananthanarayan R, Jayaram Paniker CK (2005) Enterobacteriaceae III: Salmonella. In: Ananthanarayan R, Jayaram Paniker CK (eds) Textbook of Microbiology, 7th edn. Orient Longman Ltd, Chennai, pp 290–304

Arora DR (2008) Salmonella. In: Arora DR, Arora B (eds) Text book of microbiology, 3rd edn. CBS Publishers and Distributors, New Delhi, pp 375–388

Arvanitidou M, Kanellou K, Vagiona DG (2005) Diversity of *Salmonella spp*. and Fungi in Northern Greek Rivers and their Correlation to Faecal Pollution Indicators. Environ Res 99:278–284

Bhattacharya SS, Das U, Choudhury BK (2011) Occurrence & antibiogram of *Salmonella* Typhi & *S.* Paratyphi A isolated from Rourkela. Orissa Indian J Med Res 133:431–433

Brooks GF, Butel SB, Morse SA (2004) Jawetz, Melnick, Adelberg's: medical Microbiology, 23rd edn. McGraw-Hill, Singapore, pp 256–260

Brown JC, Thomson CJ, Amyes SGB (1996) Mutations of the *gyrA* gene of clinical isolates of *Salmonella Typhimurium* and three other Salmonella species leading to decreased susceptibilities to 4-quinolone drugs. J Antimicrob Chemother 7:351–356

Cabral JPS (2010) Water microbiology. Bacterial pathogens and water. Int J Environ Res Public Health 7:3657–3703

Casadesús J (2011) Salmonella: from basic science to clinical issues. Future Microbiol 6:133–135

Chandel DS, Chaudhry R, Dhawan B, Pandey A, Dey AB (2000) Drug-resistant *Salmonella* enteric a serotype Paratyphi A in India. Emerg Infect Dis 6:420–421

Chuang CH, Su LH, Perera J, Karlos J, Tan BH, Kumarasinghe G et al (2009) Surveillance of antimicrobial resistance in *Salmonella enterica* serotype Typhi in seven Asian countries. Epidemiol Infect 137:266–269

Chuttani CS, Prakash K, Gupta P, Grover V, Kumar A (1977) Controlled field trial of a high-dose oral killed typhoid vaccine in India. Bull World Health Org 55:643–644

Crump JA, Barrett TJ, Nelson JT, Angulo FJ (2003) Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi salmonellae. Clin Infect Dis 37:75–81

Crump JA, Kretsinger K, Gay K, Hoekstra RM, Vugia DJ, Hurd S et al (2008) Clinical response and outcome of infection with *Salmonella enterica* serotype Typhi with decreased susceptibility to fluoroquinolones: a United States FoodNet multicenter retrospective cohort study. Antimicrob Agents Chemother 52:1278–1284

Crump JA, Luby SP, Mintz ED (2004) The global burden of typhoid fever. Bull World Health Org 82:346–353

Daigle F (2008) Typhi genes expressed during infection or involved in pathogenesis. J Infect Developing Countries 2:431–437

Earampamoorthy S, Koff RS (1975) Health hazards of bivalve-mollusk ingestion. Ann Intern Med 83:107–110

Edelman R, Levine MM (1986) Summary of an international workshop on typhoid fever. Rev Infect Dis 8:329–349

Effa EE, Bukirwa H (2008) Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). Cochrane Database of Systematic Reviews; 4 Art. No.: CD006083

Fenwick A (2006) Waterborne diseases: could they be consigned to history? Science 313:1077–1081

Giannella RA (2010) Infectious enteritis and proctocolitis and bacterial food poisoning. In: Feldman M, Friedman LS, Brandt LJ, (eds) Sleisenger & Fordtran's gastrointestinal and paratyphoid fever liver disease, 9th ed. Saunders Elsevier, Philadelphia: chapter 107

Gupta SK, Medalla F, Omondi MW, Whichard JM, Fields PI, Gerner Smidt P (2008) Laboratory-based surveillance of in the United States: travel and antimicrobial resistance. Clin Infect Dis 46:1656–1663

Gupta V, Kaur J, Kaistha N (2009) Re-emerging chloramphenicol sensitivity and emerging low level ciprofloxacin resistance among *Salmonella enterica* serotype Typhi isolates in North India. Trop Doct 39:28–30

House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay PV (2001) Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. J Clin Microbiol 39:1002–1007

Kanungo S, Dutta S, Sur D (2008) Epidemiology of typhoid and paratyphoid fever in India. J Infect Developing Countries 2:454–460

Keusch GT (1998) Salmonellosis. In: Harrison's principles of internal medicine, 14th edn, Vol 1. McGraw, USA, pp 950–956

Le Minor LE (2003) The genus *Salmonella*. In Dworkin M, Falkow S, Rosenberg E (eds) The Prokaryotes: an evolving electronic resource for the microbiological community, electronic release 3.14, 3rd edn. Springer, New York

Levine MM, Tacket CO, Sztein MB (2001) Host-Salmonella interaction: human trials. Microbes Infect 3:1271–1279

Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, Cryz S (1999) Duration of efficacy of Ty21a, attenuated *Salmonella* typhi live oral vaccine. Vaccine 17:S22–S27

Levine M .2001. Centre for Vaccine Development, UMB, 9th Conf on diarr and nutrition :17-18

Lima AA, Guerrant RL (2009) Inflammatory enteritides. In: Mandell GL, Bennett JE, Dolin R (eds) Principles and practice of infectious diseases, 7th edn. Elsevier Churchill Livingston, chapter 97

Lynch MF, Blanton EM, Bulens S, Polyak C, Vojdani J, Stevenson J et al (2009) Typhoid fever in the United States, 1999–2006. JAMA 302:8

Maskey AP, Basnyat B, Thwaites GE, Campbell JI, Farrar JJ, Zimmerman MD (2008) Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993–2003. Trans Royal Soc Trop Med Hyg 102:91–95

Mweu E, English M (2008) Typhoid fever in children in Africa. Trop Med Int Health 13:1–9

Nordmann P, Poirel L, Mak JK, White PA, McIver CJ, Taylor P (2008) Multidrug-resistant *Salmonella* strains expressing emerging antibiotic resistance determinants. Clin Infect Dis 46:324–325

Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD et al Domi Typhoid Study Group (2008) A study of typhoid fever in five Asian countries:disease burden and implications for control. Bull World Health Organ 86:260–268

Parry CM, Hien TT, Dougan G, Whitw NJ, Farrar JJ (2002) Typhoid fever. N Engl J Med 347:1770–1782

Parry CM, Threlfall EJ (2008) Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr Opin Infect Dis 21:531–538

Pathania NS, Sachar RS (1965) Typhoid and paratyphoid fever in Punjab (India): a study of 340 cases. Am J Trop Med Hyg 14:419–423

Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR (2006) Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis 10:434–438

Popoff MY, Le Minor LE (2005) Genus *Salmonella*. In Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology, Vol 2, Part B, 2nd edn. Springer, New York, pp 764–799

Rodrigues C, Shenai S, Mehta A (2003) Enteric fever in Mumbai, India: the good news and the bad news. Clinical Infect Dis 36:535

Rowe B, Ward LR, Threlfall EJ (1997) Multidrug-resistant *Salmonella* Typhi: a worldwide epidemic. Clin Infect Dis 24:S106–S109

Shaw AV, Reddy EA, Crump JA (2008) Etiology of community-acquired bloodstream infections in Africa. 46th Annual Meeting of the Infectious Diseases Society of America. Infectious Diseases Society of America, Washington, DC

Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, Rao M, Naficy A, Clemens JD, Bhan MK (1999) Typhoid fever in children aged less than 5 years. Lancet 354:734–737

Sood S, Kapil A, Dash N, Das BK, Goel V, Seth P (1999) Paratyphoid fever in India: an emerging problem. Emerg Infect Dis 5:483–484

Sur D, von Seidlein L, Manna B, Dutta S, Deb AK, Sarkar BL et al (2006) The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community based study. Trans R Soc Trop Med Hyg 100:725–733

Sur D, Ali M, Von SL, Manna B, Deen JL, Acosta CJ et al (2007) Comparisons of predictors for typhoid and paratyphoid fever in Kolkata. India. BMC Public Health 7:289

Swaminathan B, Beebe J, Besser J (2005) Investigation of foodborne and waterborne disease outbreaks. In Manual of Clinical Microbiology, 7th edn. American Society of India, Washington, pp 174–190

Thiem VD, Lin FYC, Canh DG, Son NH, Anh DD, Mao ND et al (2011) The Vi Conjugate Typhoid Vaccine Is Safe, Elicits Protective Levels of IgG Anti-Vi, and Is Compatible with Routine Infant Vaccines. Clin Vaccine Immunol 18:730–735

Threlfall EJ, Ward LR, Skinner JA, Smith HR, Lacey S (1999) Ciprofloxacin-resistant *Salmonella* Typhi and treatment failure. Lancet 353:1590–1591

Walia M, Gaind R, Paul P, Mehta R, Aggarwal P, Kalaivani M et al (2006) Age related clinical and microbiological characteristics of enteric fever in India. Trans R Soc Trop Med Hyg 100:942–948

Udeze AO, Abdulrehman F, Okonko IO, Anibijubon II (2010) Seroprevelence of salmonella typhi and salmonella paratyphi among the first year student of university of llorin, Nigeria. Middle East J Sci Res 6:257–262

Zaki SA, Karande S (2011) Multidrug-resistant typhoid fever: a review. J Infect Developing Countries 5:324–337