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Background document: The diagnosis, treatment and prevention of typhoid fever

Communicable Disease Surveillance and Response
Vaccines and Biologicals

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This document contains general background information on the epidemiology, infection, diagnosis, treatment and prevention of typhoid fever. It is targeted at public health professionals, clinicians and laboratory specialists. A second document to be published later, targeting health professionals in the field, will focus on the practical aspects of epidemic preparedness and the treatment of the disease.

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Abbreviations

Ig	immunoglobulin (IgG, IgM)
i.m.	intramuscular
i.v.	intravenous
LPS	lipopolysaccharide
MDR	multi-drug resistant
Mp	macrophages
NARST	nalidixic-acid-resistant *Salmonella typhi*
Vi	virulent (antigen)

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Chapter 1: The organism, the disease and transmission

1.1 The organism

Typhoid fever is caused by *Salmonella typhi*, a Gram-negative bacterium. A very similar but often less severe disease is caused by *Salmonella* serotype *paratyphi* A. The nomenclature for these bacteria is confused because the criteria for designating bacteria as individual species are not clear. Two main views on the nomenclature of the genus *Salmonella* have been discussed. Le Minor and Popoff suggested that two species should be recognized: *Salmonella bongori* and *Salmonella enterica*. *S. enterica* included six subspecies, of which subspecies I (one) contained all the pathogens of warm-blooded animals. *S. typhi* was a serotype within subspecies I: *Salmonella enterica* subspecies I serotype *typhi*. This proposal was rejected by the International Judicial Commission because the name was not well known to clinicians and its use might cause accidents endangering health or life. The original rules therefore remain in force. Ezaki and colleagues have noted in the International Journal of Systematic and Evolutionary Microbiology that the correct nomenclature for the causal agent of typhoid fever is *Salmonella typhi* and have requested that the current subspecific status of serotype *paratyphi* A should be raised to specific status, i.e. *Salmonella paratyphi A*.

S. typhi has several unique features, the genetic basis of many of which is known as a result of early genetic studies and the recent sequencing of the whole genome. Although many genes are shared with *E. coli* and at least 90% with *S. typhimurium*, there are several unique clusters of genes known as pathogenicity islands and many more single genes that seem to have been acquired by *S. typhi* during evolution. *S. typhi* can be identified in the laboratory by several biochemical and serological tests (see Chapter 2). One of the most specific is that of polysaccharide capsule Vi, which is present in about 90% of all freshly isolated *S. typhi* and has a protective effect against the bactericidal action of the serum of infected patients. This capsule provides the basis for one of the commercially available vaccines (see Chapter 4). Vi antigen is present in some other bacteria (*Citrobacter freundii*, *Salmonella paratyphi C* and *Salmonella dublin*) but not in exactly the same genetic context. The ratio of disease caused by *S. typhi* to that caused by *S. paratyphi* is about 10 to 1 in most of the countries where this matter has been studied.

1.2 The disease

During an acute infection, *S. typhi* multiplies in mononuclear phagocytic cells before being released into the bloodstream. After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages (Mp) that ingest the bacilli but do not generally kill them. Some bacilli remain within Mp of the small intestinal

lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by Mp. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of 8 to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors. Incubation periods ranging from 3 days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (~1–10 bacteria per ml of blood).

1.2.1 Symptoms

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications. Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculum ingested, host factors (e.g. HLA type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S. typhi* and *S. paratyphi* (1). Evidence of *Helicobacter pylori* infection also represents an increased risk of acquiring typhoid fever.

- Acute non-complicated disease: Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever, up to 25% of patients show exanthem (rose spots), on the chest, abdomen and back.

- Complicated disease: Acute typhoid fever may be severe. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients, and up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases. Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen.

Altered mental status in typhoid patients has been associated with a high case-fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic symptoms, although rare, have been reported. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular

coagulation, thrombocytopenia and haemolytic uraemic syndrome. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic carrier state may develop.

- Carrier state: 1–5% of patients, depending on age, become chronic carriers harbouring *S.typhi* in the gallbladder.

1.2.2 Magnitude of the problem

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries. These factors are believed to result in many cases going undiagnosed. On the basis of the literature (2, 3) and the incidence of typhoid fever recorded in control groups in large vaccine field trials with good laboratory support it has been estimated that approximately 17 million cases of typhoid fever and 600 000 associated deaths occur annually (4). However, the estimates have been biased because study populations have usually been in areas of high incidence. Furthermore, these estimates of burden relate to the clinical syndrome of typhoid fever but not to *S. typhi* exposure. Since the prevalence of bacteraemia in febrile children is quite high (2–3%) in areas of endemicity it is suggested that exposure to the bacteria is higher than indicated by the figures that are based solely on the clinical syndrome of typhoid fever. The incidence of the disease in areas of endemicity may resemble the incidences observed in control groups in large vaccine field trials, viz. between 45 per 100 000 per year and over 1000 per 100 000 per year. Preliminary results from recent studies conducted in Bangladesh by ICDDR,B show an incidence of approximately 2000 per 100 000 per year. Typhoid fever also has a very high social and economic impact because of the hospitalization of patients with acute disease and the complications and loss of income attributable to the duration of the clinical illness (5). It is important to note that reports from some provinces in China and Pakistan have indicated more cases of paratyphoid fever caused by *S. paratyphi* A than by *S. typhi*.

In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 19 years. In 1997, for example, this age range was reported during an epidemic of the disease in Tajikistan. Nevertheless, clinically apparent bacteraemic *S. typhi* infection in children aged under three years has been described in Bangladesh, India, Jordan, Nigeria, and elsewhere (6, 7). In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths. In Indonesia, people aged 3–19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year. A similar situation was reported from Papua New Guinea. When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children aged under 3 years was low. In Chile, however, single blood cultures for all children aged under 24 months who presented at health centres with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S. typhi* or *S. paratyphi* (8). Enteric fever had not been suspected on clinical grounds in any of the children. In South America the peak incidence occurred in school students aged 5–19 years and in adults aged over 35 years. This kind of study has not been conducted in other areas of endemicity.

Between 1% and 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen. The propensity to become a carrier follows the epidemiology of gall bladder disease, increasing with age and being greater in females than in males. The propensity to become a chronic carrier may have changed with the present availability and selection of antibiotics as well as with the antibiotic resistance of the prevalent strains. The role of chronic carriers as a reservoir of infection was studied in Santiago, Chile, where a crude rate of 694 carriers per 100 000 inhabitants was found (9).

1.2.3 Case definition

Confirmed case of typhoid fever

A patient with fever (38°C and above) that has lasted for at least three days, with a laboratory-confirmed positive culture (blood, bone marrow, bowel fluid) of *S. typhi.*

Probable case of typhoid fever

A patient with fever (38°C and above) that has lasted for at least three days, with a positive serodiagnosis or antigen detection test but without *S. typhi* isolation.

Chronic carrier

Excretion of *S. typhi* in stools or urine (or repeated positive bile or duodenal string cultures) for longer than one year after the onset of acute typhoid fever. Short-term carriers also exist but their epidemiological role is not as important as that of chronic carriers. Some patients excreting *S. typhi* have no history of typhoid fever.

1.3 Contamination and transmission

Humans are the only natural host and reservoir. The infection is transmitted by ingestion of food or water contaminated with faeces. Ice cream is recognized as a significant risk factor for the transmission of typhoid fever. Shellfish taken from contaminated water, and raw fruit and vegetables fertilized with sewage, have been sources of past outbreaks. The highest incidence occurs where water supplies serving large populations are contaminated with faeces. Epidemiological data suggest that waterborne transmission of *S. typhi* usually involves small inocula, whereas foodborne transmission is associated with large inocula and high attack rates over short periods. The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period. In volunteers who ingested 10^9 and 10^8 pathogenic *S. typhi* in 45 ml of skimmed milk, clinical illness appeared in 98% and 89% respectively. Doses of 10^5 caused typhoid fever in 28% to 55% of volunteers, whereas none of 14 persons who ingested 10^3 organisms developed clinical illness. Although it is widely believed that *Salmonella* is transmitted via the oral route, the transmission of *S. typhimurium* via the respiratory route has been demonstrated in a mouse model (10).

Family studies were conducted in Santiago, Chile, during an era of high typhoid endemicity in order to ascertain whether chronic carriers were significantly more frequent in households where there were index cases of children with typhoid fever than in matched control households. Other epidemiological studies investigated whether

risk factors could be identified for persons with typhoid fever in comparison with uninfected household members. It was concluded that chronic carriers in households did not play an important role in transmission. Subsequently, it was shown that the irrigation of salad with wastewater contaminated with sewage was the key factor responsible for maintaining the high endemicity of typhoid in Santiago. In developed countries, on the other hand, typhoid is transmitted when chronic carriers contaminate food as a consequence of unsatisfactory food-related hygiene practices.

Chapter 2: Diagnosis of typhoid fever

The definitive diagnosis of typhoid fever depends on the isolation of *S. typhi* from blood, bone marrow or a specific anatomical lesion. The presence of clinical symptoms characteristic of typhoid fever or the detection of a specific antibody response is suggestive of typhoid fever but not definitive. Blood culture is the mainstay of the diagnosis of this disease.

Although ox bile medium (Oxgall) is recommended for enteric fever pathogens (*S. typhi* and *S. paratyphi*), only these pathogens can be grown on it. In a general diagnostic laboratory, therefore, where other pathogens are suspected, a general blood culture medium should be used. More than 80% of patients with typhoid fever have the causative organism in their blood. A failure to isolate the organism may be caused by several factors: (i) the limitations of laboratory media (11); (ii) the presence of antibiotics (12); (iii) the volume of the specimen cultured (13); or (iv) the time of collection, patients with a history of fever for 7 to 10 days being more likely than others to have a positive blood culture. Bone marrow aspirate culture is the gold standard for the diagnosis of typhoid fever (14, 15, 16) and is particularly valuable for patients who have been previously treated, who have a long history of illness and for whom there has been a negative blood culture with the recommended volume of blood (17). Duodenal aspirate culture has also proved highly satisfactory as a diagnostic test (18) but has not found widespread acceptance because of poor tolerance of duodenal aspiration, particularly in children (19).

2.1 Specimens

If a bacteriology laboratory is not available on site, clinical specimens for culture can be transported to a main laboratory for processing. For blood culture it is essential to inoculate media at the time of drawing blood. For other specimens it is advisable to make the time of transportation to the laboratory as short as possible. It is more important to process the specimens quickly than to keep them cold. Once they have been inoculated, blood culture bottles should not be kept cold. They should be incubated at 37°C or, in tropical countries, left at room temperature, before being processed in the laboratory.

2.1.1 Blood

The volume of blood cultured is one of the most important factors in the isolation of *S. typhi* from typhoid patients: 10–15 ml should be taken from schoolchildren and adults in order to achieve optimal isolation rates; 2–4 ml are required from toddlers and preschool children (13, 17). This is because children have higher levels of bacteraemia than adults. In some regions it may be impossible to collect such large volumes of

blood and so alternative diagnostic methods may be necessary for cases in which blood cultures are negative. Because reducing the blood volume reduces the sensitivity of the blood culture, however, an effort should be made to draw sufficient blood if at all possible. Blood should be drawn by means of a sterile technique of venous puncture and should be inoculated immediately into a blood culture bottle with the syringe that has been used for collection.

Several reports of pseudobacteraemia have been associated with the reinoculation of blood culture bottles after the collection of blood in contaminated vessels. The practice of inoculating blood culture bottles from specimens taken for biochemical or haematological analysis should therefore be avoided. The optimum ratio of the volume of blood to traditional culture broth should be 1 to 10 or more (e.g. 1:12). Some commercial blood culture systems have special resins in the media which allow higher volumes of blood to be used. The instructions with commercial blood culture systems should always be read and the recommended amounts should not be exceeded. In general, if 5 ml of blood are drawn they should be inoculated into 45 ml or more of broth. If 10–15 ml of blood are drawn the specimen can be divided into equal aliquots and inoculated into two or more blood culture bottles. This allows the use of standard blood culture bottles of 50 ml. For small children the volume of blood drawn can be reduced but should still be inoculated into 45 ml of culture broth. In order to assist the interpretation of negative results the volume of blood collected should be carefully recorded. The blood culture bottle should then be transported to the main laboratory at ambient temperature (15°C to 40°C) as indicated above. Blood cultures should not be stored or transported at low temperatures. If the ambient temperature is below 15°C it is advisable to transport blood cultures in an incubator. In the laboratory, blood culture bottles should be incubated at 37°C and checked for turbidity, gas formation and other evidence of growth after 1, 2, 3 and 7 days. For days 1, 2 and 3, only bottles showing signs of positive growth are cultured on agar plates. On day 7 all bottles should be subcultured before being discarded as negative.

2.1.2 Serum

For serological purposes, 1–3 ml of blood should be inoculated into a tube without anticoagulant. A second sample, if possible, should be collected at the convalescent stage, at least 5 days later. After clotting has occurred the serum should be separated and stored in aliquots of 200 ml at +4°C. Testing can take place immediately or storage can continue for a week without affecting the antibody titre. The serum should be frozen at -20°C if longer-term storage is required.

2.1.3 Stool samples

Stools can be collected from acute patients and they are especially useful for the diagnosis of typhoid carriers. The isolation of *S. typhi* from stools is suggestive of typhoid fever. However, the clinical condition of the patient should be considered. Stool specimens should be collected in a sterile wide-mouthed plastic container. The likelihood of obtaining positive results increases with the quantity of stools collected. Specimens should preferably be processed within two hours after collection. If there is a delay the specimens should be stored in a refrigerator at 4°C or in a cool box with freezer packs, and should be transported to the laboratory in a cool box. Stool culture may increase the yield of culture-positive results by up to 5% in acute typhoid fever. If a stool sample cannot be obtained, rectal swabs inoculated into Carry Blair transport medium can been used but these are less successful.

2.2 Microbiological procedures

2.2.1 Blood culture

A typical blood culture bottle contains 45 ml of tryptic soy broth or brain heart infusion broth. These are inoculated with 5 ml of fresh blood and incubated at 37°C. Negatives should be kept for at least seven days. Because *S. typhi* is not the only bacterial pathogen found in blood, subculturing is performed on days 1, 2, 3 and 7 on non-selective agar. The best agar is blood agar (horse or sheep blood) as this allows the growth of most bacterial pathogens. If blood agar is not available, nutrient agar can be used in combination with MacKonkey agar. In some laboratories the use of MacConkey agar alone is preferred as this allows the growth of only bile-tolerant bacteria such as *S. typhi* and does not allow the growth of many Gram-positive contaminants. The contamination of blood cultures reduces isolation rates for *S. typhi* and should be prevented as far as possible. It is important to identify contaminating bacteria that come from the skin of patients or the air of the laboratory so that measures can be taken to prevent further problems. MacKonkey agar should therefore not be used as the only agar for the sampling of blood cultures in a diagnostic microbiology laboratory. Furthermore, because it is selective, MacKonkey agar does not permit the growth of Gram-positive pathogens or even all *E. coli.*

For suspected tyhoid fever, subculture plates should be incubated at 37°C for 18–24 hours in an aerobic incubator.

2.2.2 Stool or rectal swab culture

This involves inoculating 1 g of stool into 10 ml of selenite F broth and incubating at 37°C for 18–48 hours. Because selenite broth is very sensitive to heat the manufacturer's instructions should be carefully followed during preparation and overheating of the broth during sterilization should be avoided. Once a batch is prepared it should be stored at 4°C. Selenite broth inhibits the motility of *E. coli* found in stools but does not kill this bacterium. A subculture of selenite broth on a selective agar is therefore made from the surface of the broth without disturbing the sediment. The choice of agar media includes Mac Conkey agar, desoxycholate citrate agar, xylose-lysine-desoxycholate agar, and hektoen enteric agar or SS (*Salmonella–Shigella*). The plate is incubated at 37°C for 24 hours. Different batches of agar plates can give slightly different colonies of *S. typhi* and it is therefore important to keep one strain of *S. typhi* for use in quality control for each batch of agar plates and selenite broth. New batches of media are inoculated with the control strain and the amount of growth and the appearance of the colonies are recorded. If *S. typhi* does not grow as well as usual in any batch of medium, discard the medium and make a fresh one.

The identification of colonies as *S. typh*i is straightforward if reagents of satisfactory quality are available. Colonies from solid media can be used for agglutination with specific antisera. Several salmonellae may share the same antigenic structure. Consequently, confirmation by means of biochemical tests is always necessary.

2.2.3 Colony characteristics

Blood agar

On blood agar, *S. typhi* and *S. paratyphi* usually produce non-haemolytic smooth white colonies.

MacConkey agar

On MacConkey agar, salmonellae produce lactose non-fermenting smooth colonies.

SS agar

On SS agar, salmonellae usually produce lactose non-fermenting colonies with black centres (except *S. paratyphi* A, whose colonies do not have black centres).

Desoxycholate agar

On desoxycholate agar, salmonellae produce lactose non-fermenting colonies with black centres (except *S. paratyphi* A, whose colonies do not have black centres).

Xylose-lysine-desoxycholate agar

On xylose-desoxycholate agar, salmonellae produce transparent red colonies with black centres (except *S. paratyphi* A, whose colonies do not have black centres).

Hektoen enteric agar

On hektoen enteric agar, salmonellae produce transparent green colonies with black centres (except *S. paratyphi* A, whose colonies do not have black centres).

Bismuth sulfite agar

On this medium, salmonellae produce black colonies.

2.2.4 Biochemical identification

Suspected colonies obtained on the above media are screened by means of the following media/tests:

Organism	Kliger's iron agar				Motility, indol urea			Citrate
	Slant	Butt	H_2S	Gas	Motility	Indol	Urea	
S. typhi	Alk	Acid	Wk+	–	+	–	–	–
S. paratyphi A	Ald	Acid	–	+	+	–	–	–
Other *Salmonella* spp.	Alk	Acid	V	V	+	–	–	V
E. coli	Acid	Acid	–	+	+	+	–	–
Klebsiella spp.	Acid	Acid	–	++	–	V	+	+
Citrobacter spp.	V	Acid	+++	+	+	V	–	+
Proteus spp.	Alk	Acid	+	+	+	V	++	V

The production of acid turns the agar yellow. For the slant this means lactose fermentation and for the butt this means glucose fermentation.

Alk = alkaline, Wk = weak, V= variable result.

2.3 Serological procedures

2.3.1 Serological identification of Salmonella

Salmonellae can be characterized by their somatic (O) and flagellar (H) antigens, the latter existing in some serotypes in phases 1 and 2. Some salmonellae also have an envelop antigen called Vi (virulence). The salmonellae that cause typhoid fever and paratyphoid fever have the following antigenic compositions and belong to the serogroups indicated.

Serotype	O antigen	H antigen	Serogroup Phase1:2
S. typhi	9, 12, (Vi)	d :	Group D1
S. paratyphi A	1, 2, 12	a : (1, 5)	Group A
S. paratyphi B	1, 4, (5), 12	b : 1, 2	Group B
S. paratyphi C	6, 7, (Vi)	c : 1, 5	Group C1

Antigens in parentheses are either weak or absent in some isolates.

The O antigen is usually determined by means of the slide agglutination test with group-specific antiserum followed by agglutination with factor antiserum. Growth from non-selective agar or Kliger's iron agar can be used for the determination of O antigen. Strains of *S. typhi* and *S. paratyphi* C may possess Vi antigen that render the strains non-agglutinable in O antisera. These cultures agglutinate in Vi antiserum.

They will agglutinate in O antiserum, however, after destruction of the Vi antigen by boiling the culture for 10 minutes. The specific O antigen is confirmed by slide agglutination with factor antiserum. The specific O antigens for typhoid fever organisms are shown below.

Organism	Specific O antigen factor
S. typhi	9
S. paratyphi A	2
S. paratyphi B	4
S. paratyphi C	6/7

H antigen is usually determined by means of the tube agglutination test. The organisms should be motile and from a liquid culture. The motility of weakly motile organisms can be enhanced by repeated passage in liquid cultures. Determination of the O antigen and the phase 1 H antigen only is usually sufficient for the identification of typhoid fever organisms and paratyphoid fever organisms. The specific phase 1 antigens for typhoid fever organisms are shown below. Antisera against these antigens are used, usually in the tube agglutination test.

Organism	Phase 1 H antigen
S. typhi	d
S. paratyphi A	a
S. paratyphi B	b
S. paratyphi C	c

In some cultures, only some organisms express phase 1 H antigens and others express phase 1 and 2 H antigens at the same time. Such cultures agglutinate with both phase 1 and phase 2 antisera. In cultures where a single phase antigen is expressed the antigen in the other phase can be induced by incubating the culture with antiserum to the phase antigen being expressed (20).

S. typhi with flagella variant Hj and with phase 2 antigen Z66 have been reported but they are rare. *Salmonella java* can be misidentified as *S. paratyyphi* B because these two serotypes have identical antigens. However, the serotypes can be differentiated biochemically. The former is tartrate-positive and produces non-typhoid salmonellosis, and the latter is tartrate-negative and produces typhoid salmonellosis. *S. java* is now considered to be a tartrate-positive variant of *S. paratyphi* B. *S. dublin* and *Citrobacter freundii* possess Vi antigen. However, the phase 1 H antigens of *S. dublin* are g, p as against d in *S. typhi*. Unlike *C. freundii*, *S. typhi* does not grow in KCN broth (21). Some of the non-typhoidal salmonellae can cause febrile illness that mimics enteric fever. However, these strains can be differentiated biochemically.

2.3.2 Felix-Widal test

This test measures agglutinating antibody levels against O and H antigens. The levels are measured by using doubling dilutions of sera in large test tubes. Usually, O antibodies appear on days 6-8 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on an acute serum (at first contact with the patient). A convalescent serum should preferably also be collected so that paired titrations can be performed. In practice, however, this is often difficult. At least 1 ml of blood should be collected each time in order to have a sufficient amount of serum. In exceptional circumstances the test can be performed on plasma without any adverse effect on the result.

The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibody response. On the other hand, *S. typhi* shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriacae, and this can lead to false-positive results. Such results may also occur in other clinical conditions, e.g. malaria, typhus, bacteraemia caused by other organisms, and cirrhosis. In areas of endemicity there is often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas (22).

It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which the antibody titre is considered significant. This is particularly important if, as usually happens, a single acute sample is available for testing. If paired sera are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic. Quality control of the test is achieved by running a standard serum with a known antibody titre in parallel in each batch of assays. The variations in the standard serum should not exceed one tube, i.e. double dilution.

Despite these limitations the test may be useful, particularly in areas that cannot afford the more expensive diagnostic methods (23). This is acceptable so long as the results are interpreted with care in accordance with appropriate local cut-off values for the determination of positivity. This test is unnecessary if the diagnosis has already been confirmed by the isolation of *S. typhi* from a sterile site. New diagnostic tests are being developed.

2.3.3 New diagnostic tests: current status and usefulness

There is a need for a quick and reliable diagnostic test for typhoid fever as an alternative to the Widal test. Recent advances include the IDL Tubex® test marketed by a Swedish company, which reportedly can detect IgM O9 antibodies from patients within a few minutes. Another rapid serological test, Typhidot®, takes three hours to perform. It was developed in Malaysia for the detection of specific IgM and IgG antibodies against a 50 kD antigen of *S. typhi*. A newer version of the test, Typhidot-M®, was recently developed to detect specific IgM antibodies only. The dipstick test, developed in the Netherlands, is based on the binding of *S. typhi*-specific IgM antibodies in samples to *S. typhi* lipopolysaccharide (LPS) antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles.

IDL Tubex® test

The Tubex® test is simple (essentially a one-step test) and rapid (taking approximately two minutes). It exploits the simplicity and user-friendliness of the Widal and the slide latex agglutination tests but uses the separation of coloured particles in solution to improve resolution and sensitivity. Specificity is improved by means of an inhibition assay format and by detecting antibodies to a single antigen in *S. typhi* only. The O9 antigen used in the test is extremely specific because its immunodominant epitope is a very rare dideoxyhexose sugar that occurs in nature. This antigen has been found in serogroup D *salmonellae* but not in other microorganisms. The closest to it is the tyvelose antigen found in *Trichinella spiralis* but antibodies to these two antigens do not cross-react with each other. A positive result given by Tubex® invariably suggests a *Salmonella* infection, although the test cannot tell which group D *Salmonella* is responsible. Infections caused by other serotypes, including *S. paratyphi* A, give negative results.

Immunogenically, the O9 antigen is immunodominant and robust. Unlike the capsular (Vi) and flagellar antigens that are thymus-independent type II in nature and poorly immunogenic in infants, the O9 antigen (or LPS in general) is thymus-independent type I, immunogenic in infants, and a potent B cell mitogen. It can stimulate B cells without the help of T cells (unlike protein antigens) and, consequently, anti-O9 responses are rapid. This is important teleologically, as they form the first line of host defence. For reasons yet to be elucidated, Tubex® detects IgM antibodies but not IgG. This makes it invaluable as an aid in the diagnosis of current infections.

The test pack includes: 1) sets of specially-designed V-shaped tubes that allow six samples per set to be examined simultaneously; 2) reagent A, comprising magnetic particles coated with *S. typhi* LPS; 3) reagent B, comprising blue-coloured latex particles coated with a monoclonal antibody specific for the O9 antigen. The reagents are stable for over a year at 4°C, and for at least some weeks at ambient temperature.

A drop of test serum is mixed for about one minute with a drop of reagent A in the tube. Two drops of reagent B are then added and the contents are mixed thoroughly for 1–2 minutes. The set of tubes is then placed on a magnet-embedded stand, across which they are slid several times. The result, which can be read immediately or up to many hours later, is based on the colour of the reaction mixture. A range of colours involving varying proportions of redness and blueness can be expected, and a colour chart is provided for the purpose of scoring. Red indicates negativity while increasing blueness denotes increasing positivity.

The rationale of the test is as follows. If the serum is negative for O9 antibodies the antibody-coated indicator particles bind to the antigen-coated magnetic beads. When a magnet is applied, the magnetic particles settle to the bottom of the tube together with any blue indicator particles associated with these. Consequently a background red colour is left in the solution. This background colour is actually exploited to camouflage the sample colour of haemolysed sera. If, on the other hand, the patient's serum contains O9 antibodies, these bind to the magnetic particles and prevent the indicator particles from binding to them. The indicator particles thus remain suspended and the resultant colour of the solution is blue.

Tubex® has not been evaluated extensively but several trials are being planned. In a preliminary study involving stored sera the test performed better than the Widal test in both sensitivity and specificity (24).

Typhidot® test

This test makes use of the 50 kD antigen to detect specific IgM and IgG antibodies to *S. typhi* (25). It has undergone full-scale multinational clinical evaluation of its diagnostic value (26, 27, 28). This dot EIA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggests acute typhoid in the middle phase of infection. In areas of high endemicity where the rate of typhoid transmission is high the detection of specific IgG increases. Since IgG can persist for more than two years after typhoid infection (29) the detection of specific IgG cannot differentiate between acute and convalescent cases. Furthermore, false-positive results attributable to previous infection may occur. On the other hand, IgG positivity may also occur in the event of current reinfection. In cases of reinfection there is a secondary immune response with a significant boosting of IgG over IgM, such that the latter cannot be detected and its effect is masked. A possible strategy for solving these problems is to enable the detection of IgM by ensuring that it is unmasked (30). In order to increase diagnostic accuracy in these situations the original Typhidot® test was modified by inactivating total IgG in the serum sample. Studies with the modified test, Typhidot-M®, have shown that inactivation of IgG removes competitive binding and allows access of the antigen to the specific IgM when it is present. The detection of specific IgM within three hours suggests acute typhoid infection. Evaluations of Typhidot® and Typhidot-M® in clinical settings showed that they performed better than the Widal test and the culture method (30).

In laboratory diagnoses of typhoid fever the method used as the gold standard should approach 100% in sensitivity, specificity and positive and negative predictive values. Evaluation studies have shown that Typhidot-M® is superior to the culture method (28). Although culture remains the gold standard it cannot match Typhidot-M® in sensitivity (>93%), negative predictive value and speed (28). Typhidot-M® can replace the Widal test when used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The high negative predictive value of the test suggests that Typhidot-M® would be useful in areas of high endemicity.

IgM dipstick test

The typhoid IgM dipstick assay is designed for the serodiagnosis of typhoid fever through the detection of *S. typhi*-specific IgM antibodies in serum or whole blood samples.

The assay consists of a dipstick, a lyophilized non-enzymatic detection reagent, liquid to reconstitute the detection reagent, liquid to wet the test strip of the dipstick before incubation with serum and detection reagent, and test tubes. The components are stable for two years if stored in the temperature range 4-25°C in a dry place and protected from direct exposure to sunlight.

The assay is based on the binding of *S. typhi*-specific IgM antibodies to *S. typhi* LPS antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles. The white test strip of the dipstick contains the antigen immobilized in a distinct line. The strip also has a control line with anti-human IgM antibodies.

The assay is performed by incubation of the wetted test strip in a mixture of serum and detection reagent, the serum being diluted at 1:50 in the detection reagent. Whole blood may be tested at a 1:25 dilution in detection reagent. The incubation period is three hours at room temperature. When incubation is complete the test strip is rinsed thoroughly with water and then allowed to dry. The result is read by visual inspection of the test strip for staining of the antigen and control lines. The test result is scored negative if no staining of the antigen line occurs and is graded 1+, 2+, 3+ or 4+ if there is weak, moderate strong or very strong staining as indicated by comparison with a coloured reference strip. The control line should stain in all runs.

Evaluations of the dipstick test in laboratory-based studies in Indonesia (31, 32), Kenya (33), Viet Nam (33) and Egypt (34) have shown consistent results. These studies indicated sensitivities of 65% to 77% for samples collected at the time of first consultation from culture-confirmed patients and specificities of 95% to 100%. The results of culture and serological investigation may be influenced by various factors, among them the time of sample collection and the use of antibiotics before consultation and sample collection. In a study conducted in Makassar, Indonesia, the sensitivity of the blood culture method was estimated to be 66%, and that of the dipstick test calculated for the combined group of culture-confirmed and culture-negative patients with a final clinical diagnosis of typhoid fever was 48%. The sensitivity ranged from 29% for samples collected during the first week of illness to 96% for samples collected at a later stage. Tests on follow-up samples showed seroconversion in the majority of the dipstick-negative typhoid patients.

The dipstick test provides a rapid and simple alternative for the diagnosis of typhoid fever, particularly in situations where culture facilities are not available. The assay can be performed by people without formal training and in the absence of specialized equipment. Electricity is not required, as the components can be stored without cooling. The results of the dipstick test can be obtained on the day when patients present. This makes prompt treatment possible. Specific antibodies usually only appear a week after the onset of symptoms and signs. This should kept in mind when a negative serological test result is being interpreted.

2.4 Antimicrobial susceptibility test for typhoid fever organisms

Antimicrobial susceptibility testing is crucial for the guidance of clinical management. Isolates from many parts of the world are now multidrug-resistant (MDR) (35, 36, 37). Isolates are usually resistant to ampicillin, chloramphenicol, sulfonamide, trimethoprim, streptomycin and tetracycline. Alternative drugs that are used for treatment include: fluoroquinolones (e.g. ciprofloxacin), third-generation cephalosporins (e.g. ceftriaxone, cefotaxime), a monobactum beta-lactam (aztreonam) and a macrolide (azithromycin). Even though resistance to the first two has been noted they nevertheless remain useful (38). Reduced susceptibility to fluoroquinolones is indicated by in vitro resistance to nalidixic acid (39).

In vitro susceptibility testing usually involves disc diffusion. The choice of antimicrobial agents for the test is dictated by the agents that are currently being used for treatment and the desire to determine the prevalence of MDR strains. After the previous first-line drugs were discontinued for the treatment of typhoid fever in Bangladesh because of the emergence of MDR strains, the prevalence of multidrug resistance decreased and the possibility arose of using these drugs again (40). It is therefore recommended that susceptibility tests be performed against the following antimicrobial agents: a fluoroquinolone, a third-generation cephalosporin and any other drug currently used for treatment, nalidixic acid (for determining reduced susceptibility to fluoroquinolones because of the possibility of false in vitro susceptibility against the fluoroquinolone used for treatment), and the previous first-line antimicrobials to which the strains could be resistant (chloramphenicol, ampicillin, trimethoprim/sulfamethoxazole, streptomycin and tetracycline). Azithromycin disc test results should be interpreted with caution. The appropriate break-point recommendations for azithromycin against *S. typhi* are still not clear. Patients may respond satisfactorily to azithromycin even if isolates are intermediate according to current guidelines.

2.5 Storage of typhoid fever organisms

The isolates can be stored for up to two to three years on a nutrient agar, e.g. trypticase soy agar. The butt is stabbed and the slant is streaked and incubation takes place at 37°C for 18–24 hours. The tube is corked and made airtight by either covering the cork with parafilm or dipping the cork in melted paraffin. Alternatively, sterile mineral oil is poured on to cover the growth in the slant and the tube is corked. The tube is stored at room temperature, preferably between 20°C and 22°C, away from light in a closed cupboard. There is a risk that plasmids encoding antimicrobial resistance or other properties can be lost from isolates stored in this way.

Isolates can be stored for several years by freeze-drying, inoculating a thick suspension of growth in a nutrient broth (e.g. trypticase soy broth) with 15% glycerol in a cryovial, 10% skim milk in a cryovial, or a cryovial with beads, and freezing the vial at -70°C. Plasmids in isolates stored by these methods are stable. The use of a cryovial with beads has the advantage that beads can be removed for subculture without thawing the culture.

2.6 Quality control

The steps involved in the accurate laboratory diagnosis of typhoid fever include specimen collection and transport, the performance of laboratory procedures, and reporting. It is important that the correct specimen is collected in the correct volume, that it is transported to the laboratory in the right condition, that correct laboratory procedures are followed and that reporting is accurate. These steps should therefore be monitored at all levels and correction should take place if unacceptable performance is identified. Quality assurance is vital to the success of such investigations.

Quality control programmes ensure that the information generated by laboratories is accurate, reliable and reproducible. This is accomplished by assessing the quality of specimens and monitoring the performance of test procedures, reagents, media, instruments, and personnel. Laboratories should have internal quality control programmes. A panel of reference isolates consisting of typhoid and non-typhoid salmonellae and other Enterobacteriaceae should be maintained. At periodic intervals,

e.g. monthly, the laboratory supervisor should submit a random selection of the reference isolates under code to laboratory technologists for evaluation. Quality control of the disc susceptibility test should take place. *E. coli* ATCC 25922 should be run in parallel with the test strains. The susceptibility zones for the reference strain against various antimicrobials should be within the acceptable ranges. The results of these evaluations should be entered in a quality control monitoring book. Appropriate measures should be taken to solve any problems that are encountered.

It is desirable to participate in external quality control programmes whenever possible. It should be noted that this is relatively expensive and that there could be problems relating to the timely transport of quality control specimens to certain countries because of uncertainties about carriers and customs.

It is important to confirm *Salmonella* isolates in a reference laboratory because of the possibility of their misidentification.

Chapter 3: Treatment of typhoid fever

3.1 General management

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-up for complications or failure to respond to therapy (41). However, patients with persistent vomiting, severe diarrhoea and abdominal distension may require hospitalization and parenteral antibiotic therapy.

3.2 Antimicrobial therapy

Efficacy, availability and cost are important criteria for the selection of first-line antibiotics to be used in developing countries. This section reviews the therapeutic guidelines for the treatment of typhoid fever across all age groups. It should be noted, however, that therapeutic strategies for children, e.g. the choice of antibiotics, the dosage regimen and the duration of therapy, may differ from those for adults.

The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults (42). They are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs, viz. chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole (Table 1). The majority of isolates are still sensitive. The fluoroquinolones attain excellent tissue penetration, kill *S. typhi* in its intracellular stationary stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs. They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days, and very low rates of post-treatment carriage (43, 44). Evidence from various settings in Asia indicates that the fluoroquinolones are equally effective in the treatment of typhoid fever in children

However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs. Resistance to the fluoroquinolones may be total or partial. The so-called nalidixic-acid-resistant *S. typhi* (NARST) is a marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. Nalidixic acid itself is never used for the treatment of typhoid. These isolates are susceptible to fluoroquinolones in disc sensitivity testing according to current guidelines. However, the clinical response to treatment with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-senstive strains. There is a significant number of MDR strains from the Indian subcontinent

and some other Asian countries (not Indonesia). *S. typhi* has recently emerged as a problem in Kenya. Nalidixic-acid-resistant strains are now endemic in many areas of Viet Nam and have also been reported from the Indian subcontinent and Tajikistan. There are disturbing recent reports of the emergence of fluorquinolone-resistant isolates in various parts of Asia (45, 46, 47) and there have been a few reports of resistance to third-generation cephalopsorins in the same region. Reassuringly, however, many of these reports are coupled with evidence of the re-emergence of sensitive isolates in the same regions. Table 1 outlines the treatment strategies for uncomplicated typhoid.

Table 1. Treatment of uncomplicated typhoid fever

	Optimal therapy			Alternative effective drugs		
Susceptibility	**Antibiotic**	**Daily dose mg/kg**	**Days**	**Antibiotic**	**Daily dose mg/kg**	**Days**
Fully sensitive	Fluoroquinolone e.g. ofloxacin or ciprofloxacin	15	5–7[a]	Chloramphenicol Amoxicillin TMP-SMX	50–75 75–100 8–40	14–21 14 14
Multidrug resistance	Fluoroquinolone or cefixime	15 15–20	5–7 7–14	Azithromycin Cefixime	8–10 15–20	7 7–14
Quinolone resistance[b]	Azithromycin or ceftriaxone	8–10 75	7 10–14	Cefixime	20	7–14

[a] Three-day courses are also effective and are particularly so in epidemic containment.

[b] The optimum treatment for quinolone-resistant typhoid fever has not been determined. Azithromycin, the third-generation cephalosporins, or a 10–14 day course of high-dose fluoroquinolones, is effective. Combinations of these are now being evaluated.

The available fluoroquinolones (ofloxacin, ciprofloxacin, fleroxacin, perfloxacin) are highly active and equivalent in efficacy (with the exception of norfloxacin which has inadequate oral bioavailability and should not be used in typhoid fever).

The fluoroquinolone drugs are generally very well tolerated. However, in some countries the use of fluoroquinolones is relatively contraindicated in children because of concerns that they may cause articular damage. These agents are not registered for routine use in children. The concerns have arisen because of evidence of articular damage in growing, weight-bearing joints in beagles (48). There is now extensive experience in the use of these drugs in large numbers of children with a variety of conditions, often with long-term follow-up (cystic fibrosis, typhoid), and in the extensive use of short courses of fluoroquinolones in children for the treatment of both typhoid fever and bacillary dysentery (49). Their considerable benefits, particularly in areas where there are no affordable oral alternatives, outweigh the putative risk. The only known articular side-effect is Achilles tendon rupture in patients who are also taking corticosteroids, and this has been reported only rarely.

Ciprofloxacin, ofloxacin, perfloxacin and fleroxacin have generally proved effective. In recent years, however, there have been many reports of reduced susceptibility and treatment failure for ciprofloxacin (50, 51). No evidence of toxicity and impact on growth has been described in children with typhoid who have received ciprofloxacin (49). There is no evidence of the superiority of any particular fluoroquinolone. Nalidixic acid and norfloxacin do not achieve adequate blood concentrations after oral

administration and should not be used. For nalidixic-acid-sensitive *S. typhi*, seven-day regimens have proved highly effective. Courses of treatment of three and five days have also proved highly effective against nalidixic-acid-sensitive strains. These very short courses are best reserved for outbreaks when antibiotics are in short supply. For nalidixic-acid-resistant infections a minimum of seven days of treatment at the maximum permitted dosage is necessary and 10–14 days are usually required. Courses shorter than seven days are unsatisfactory.

Chloramphenicol, despite the risk of agranulocytosis in 1 per 10 000 patients, is still widely prescribed in developing countries for the treatment of typhoid fever (52, 53, 54). *S. typhi* strains from many areas of the world, e.g. most countries in Africa and Asia, remain sensitive to this drug and it is widely available in most primary care settings in developing countries for the treatment of pneumonia.

The disadvantages of using chloramphenicol include a relatively high rate of relapse (5–7%), long treatment courses (14 days) and the frequent development of a carrier state in adults. The recommended dosage is 50–75 mg per kg per day for 14 days divided into four doses per day (54), or for at least five to seven days after defervescence. The usual adult dose is 500 mg given four times a day. Oral administration gives slightly greater bioavailability than intramuscular (i.m.) or intravenous (i.v.) administration of the succinate salt.

Ampicillin and amoxicillin are used at 50 to 100 mg per kg per day orally, i.m. or i.v., divided into three or four doses. No benefit has been reported to result from the addition of clavulanic acid to amoxicillin.

Trimethoprim-sulfamethoxazole, (TMP–SMZ) can be used orally or i.v. in adults at a dose of 160 mg TMP plus 800 mg SMZ twice daily or in children at 4 mg TMP per kg and 20 mg SMZ per kg for 14 days (55).

Of the third-generation cephalosporins, oral cefixime (15–20 mg per kg per day for adults, 100–200 mg twice daily) has been widely used in children in a variety of geographical settings and found to be satisfactory (56, 57, 58). However, a trial of cefixime in MDR typhoid in Viet Nam indicated significantly higher treatment failure rates than with ofloxacin (59). Other agents, e.g. cefodoxime, have proved successful against typhoid fever (60). Because of the rising rates of quinolone resistance (61) there is a clear need to identify improved strategies for treating MDR typhoid in childhood. Recent data on the use of azithromycin in children indicate that it may be safely given as an alternative agent for the treatment of uncomplicated typhoid fever (62).

Azithromycin in a dose of 500 mg (10 mg/kg) given once daily for seven days has proved effective in the treatment of typhoid fever in adults and children with defervescence times similar to those reported for chloramphenicol. A dose of 1 g per day for five days was also effective in adults (42).

If intravenous antibiotics are required, i.v. cephalosporins can be given in the following doses: ceftriaxone, 50–75 mg per kg per day (2–4 g per day for adults) in one or two doses; cefotaxime, 40–80 mg per kg per day (2–4 g per day for adults) in two or three doses; and cefoperazone, 50-100 mg per kg per day (2–4 g per day for adults) in two doses. Ciprofloxacin, ofloxacin and pefloxacin are also available for i.v. use.

There are few data on the treatment of typhoid in pregnancy. The beta-lactams are considered safe (63). There have been several case reports of the successful use of fluoroquinolones but these have generally not been recommended in pregnancy because of safety concerns (64, 65). Ampicillin is safe in pregnant or nursing women, as is ceftriaxone in such women with severe or MDR disease. Although there are no data indicating that azithromycin is unsafe for pregnant or nursing women, alternatives should be used if available.

Most of the data from randomized controlled trials relate to patients treated in regions of endemicity. There are few data from such trials relating to patients treated in regions where the disease is not endemic or to returning travellers. Knowledge of the antibiotic sensitivity of the infecting strain is crucial in determining drug choice. If no culture is available a knowledge of likely sensitivity as indicated by the available global data may be useful.

The evidence suggests that the fluoroquinolones are the optimal choice for the treatment of typhoid fever in adults and that they may also be used in children. The recent emergence of resistance to fluoroquinolones, however, suggests that their widespread and indiscriminate use in primary care settings should be restricted. In areas of the world where the fluoroquinolones are not available or not registered for public health use and where the bacterium is still fully sensitive to traditonal first-line drugs (chloramphenicol, amoxicillin or trimethoprim-sulfamethoxazole), these remain appropriate for the treatment of typhoid fever. They are inexpensive, widely available and rarely associated with side-effects.

3.3 Management of complications

Both outpatients and inpatients with typhoid fever should be closely monitored for the development of complications. Timely intervention can prevent or reduce morbidity and mortality. The parenteral fluoroquinolones are probably the antibiotics of choice for severe infections but there have been no randomized antibiotic trials (66). In severe typhoid the fluoroquinolones are given for a minimum of 10 days (Table 2). Typhoid fever patients with changes in mental status, characterized by delirium, obtundation and stupor, should be immediately evaluated for meningitis by examination of the cerebrospinal fluid. If the findings are normal and typhoid meningitis is suspected, adults and children should immediately be treated with high-dose intravenous dexamethasone in addition to antimicrobials (67). If dexamethasone is given in an initial dose of 3 mg/kg by slow i.v. infusion over 30 minutes and if, after six hours, 1 mg/kg is administered and subsequently repeated at six-hourly intervals on seven further occasions, mortality can be reduced by some 80–90% in these high-risk patients. Hydrocortisone in a lower dose is not effective (68). High-dose steroid treatment can be given before the results of typhoid blood cultures are available if other causes of severe disease are unlikely.

Table 2. Treatment of severe typhoid fever

	Optimal parenteral drug			Alternative effective parenteral drug		
Susceptibility	**Antibiotic**	**Daily dose mg/kg**	**Days**	**Antibiotic**	**Daily dose mg/kg**	**Days**
Fully sensitive	Fluoroquinolone e.g. ofloxacin	15	10–14	Chloramphenicol Amoxicillin TMP-SMX	100 100 8–40	14–21 14 14
Multidrug resistant	Fluoroquinolone	15	10–14	Ceftriaxone or cefotaxime	60 80	10–14
Quinolone resistant	Ceftriaxone or cefotaxime	60 80	10–14	Fluoroquinolone	20	7–14

Patients with intestinal haemorrhage need intensive care, monitoring and blood transfusion. Intervention is not needed unless there is significant blood loss. Surgical consultation for suspected intestinal perforation is indicated. If perforation is confirmed, surgical repair should not be delayed longer than six hours. Metronidazole and gentamicin or ceftriazone should be administered before and after surgery if a fluoroquinolone is not being used to treat leakage of intestinal bacteria into the abdominal cavity. Early intervention is crucial, and mortality rates increase as the delay between perforation and surgery lengthens. Mortality rates vary between 10% and 32% (69).

Relapses involving acute illness occur in 5–20% of typhoid fever cases that have apparently been treated successfully. A relapse is heralded by the return of fever soon after the completion of antibiotic treatment. The clinical manifestation is frequently milder than the initial illness. Cultures should be obtained and standard treatment should be administered. In the event of a relapse the absence of schistosomiasis should be confirmed.

3.4 Management of carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for *S. typhi* a year following recovery from acute illness. Overall, some 1–5% of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, patients older than 50 years, and patients with cholelithiasis or schistosomiasis. If cholelithiasis or schistosomiasis is present the patient probably requires cholecystectomy or antiparasitic medication in addition to antibiotics in order to achieve bacteriological cure. In order to eradicate *S. typhi* carriage, amoxicillin or ampicillin (100 mg per kg per day) plus probenecid (Benemid®) (1 g orally or 23 mg per kg for children)or TMP-SMZ (160 to 800 mg twice daily) is administered for six weeks; about 60% of persons treated with either regimen can be expected to have negative cultures on follow-up. Clearance of up to 80% of chronic carriers can be achieved with the administration of 750 mg of ciprofloxacin twice daily for 28 days or 400 mg of norfloxacin. Other quinolone drugs may yield similar results (70, 71).

Carriers should be excluded from any activities involving food preparation and serving, as should convalescent patients and any persons with possible symptoms of typhoid fever. Although it would be difficult for typhoid carriers in developing countries to follow this recommendation, food handlers should not resume their duties until they have had three negative stool cultures at least one month apart.

Vi antibody determination has been used as a screening technique to identify carriers among food handlers and in outbreak investigations. Vi antibodies are very high in chronic *S. typhi* carriers (72).

Chapter 4:
Prevention of typhoid fever

The major routes of transmission of typhoid fever are through drinking water or eating food contaminated with *Salmonella typhi*. Prevention is based on ensuring access to safe water and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour change.

4.1 Safe water

Typhoid fever is a waterborne disease and the main preventive measure is to ensure access to safe water. The water needs to be of good quality and must be sufficient to supply all the community with enough drinking water as well as for all other domestic purposes such as cooking and washing.

During outbreaks the following control measures are of particular interest:

- **In urban areas**, control and treatment of the water supply systems must be strengthened from catchment to consumer. Safe drinking water should be made available to the population trough a piped system or from tanker trucks.

- **In rural areas**, wells must be checked for pathogens and treated if necessary.

- **At home**, a particular attention must be paid to the disinfection and the storage of the water however safe its source. Drinking-water can be made safe by boiling it for one minute or by adding a chlorine-releasing chemical. Narrow-mouthed pots with covers for storing water are helpful in reducing secondary transmission of typhoid fever. Chlorine is ineffective when water is stored in metallic containers.

- **In some situations**, such as poor rural areas in developing countries or refugee camps, fuel for boiling water and storage containers may have to be supplied.

4.2 Food safety

Contaminated food is another important vehicle for typhoid fever transmission.

Appropriate food handling and processing is paramount and the following basic hygiene measures must be implemented or reinforced during epidemics:

- washing hands with soap before preparing or eating food;

- avoiding raw food, shellfish, ice;

- eating only cooked and still hot food or re-heating it.

During outbreaks, food safety inspections must be reinforced in restaurants and for street food vendors activities .

Typhoid can be transmitted by chronic carriers who do not apply satisfactory food-related hygiene practices. These carriers should be excluded from any activities involving food preparation and serving. They should not resume their duties until they have had three negative stool cultures at least one month apart.

4.3 Sanitation

Proper sanitation contributes to reducing the risk of transmission of all diarrhoeal pathogens including *Salmonella typhi*.

- Appropriate facilities for human waste disposal must be available for all the community. In an emergency, pit latrines can be quickly built.

- Collection and treatment of sewage, especially during the rainy season, must be implemented

- In areas where typhoid fever is known to be present, the use of human excreta as fertilisers must be discouraged.

4.4 Health education

Health education is paramount to raise public awareness on all the above mentioned prevention measures. Health education messages for the vulnerable communities need to be adapted to local conditions and translated into local languages. In order to reach communities, all possible means of communication (e.g. media, schools, women's groups, religious groups) must be applied.

Community involvement is the cornerstone of behaviour change with regard to hygiene and for setting up and maintenance of the needed infrastructures.

In health facilities, all staff must be repeatedly educated about the need for :

- excellent personal hygiene at work;

- isolation measures for the patient;

- disinfection measure.

4.5 Vaccination

4.5.1 Currently available vaccines

The old parenteral killed whole-cell vaccine was effective but produced strong side-effects because of LPS. Two safe and effective vaccines are now licensed and available. One is based on defined subunit antigens, the other on whole-cell live attenuated bacteria.

The first of these vaccines, containing Vi polysaccharide, is given in a single dose subcutaneous (s.c.) or i.m. Protection begins seven days after injection, maximum protection being reached 28 days after injection when the highest antibody concentration is obtained. In field trials conducted in Nepal and South Africa, where the disease is endemic and attack rates reach 900/100 000, the protective efficacy was 72% one and half years after vaccination (74) and was still 55% three years after a single dose (75). The vaccine is approved for persons aged over two years. Revaccination is recommended every three years for travellers. In a field trial in South Africa, 10 years after immunization 58% of vaccinees still had over 1 ìg/ml of anti-Vi antibodies in their blood (76), i.e. a protective level. In efficacy trials conducted in Chiang Su and Guangxi, China, in 1995 and 1997 respectively with a locally produced Vi vaccine, 72% protection was obtained in vaccinees (77, 78). A protective efficacy of 70% was reported in a population vaccinated before or during an outbreak situation in the same country (78). The Vi vaccine is licensed in Australia and in more than 92 countries in Africa, the Americas, Asia, and Europe. It is mainly used by travellers visiting areas at high risk of typhoid fever because of the presence of multidrug-resistant strains. There have been a few reports of Vi-negative *S. typhi* strains (79). However, *S. typhi* strains freshly isolated from the blood of patients have always been Vi-positive. During laboratory storage or transfer the Vi capsule may be lost but even if this happens through gene mutation or alteration it is quite uncommon. Moreover, this is not a major problem in relation to the protection obtained in Asian countries where Vi-negative strains have been reported at the low average level of 3%. The majority of the 600 000 estimated deaths per year are in Asia. Vaccinated people with Vi can be differentiated from *S. typhi* carriers because of the higher level of Vi antibodies in the latter (see 3.4 above).

The live oral vaccine Ty2la is available in enteric-coated capsule (80) or liquid formulation. It should be taken in three doses two days apart on an empty stomach. It elicits protection as from 10–14 days after the third dose. It is approved for use in children aged at least 5 years. Travellers should be revaccinated annually. The protective efficacy of the enteric-coated capsule formulation seven years after the last dose is still 62% in areas where the disease is endemic; the corresponding figure for the liquid formulation is 70%. Herd immunity was clearly demonstrated during field trials in Chile. Antibiotics should be avoided for seven days before or after the immunization series. This vaccine is licensed in 56 countries in Africa, Asia, Europe, South America, and the USA. Although the package insert allows simultaneous administration of mefloquine (Lariam®) or chloroquine (Nivaquine® or Aralen®) for malaria prophylaxis, it is recommended that an interval of three days be maintained between the completion of the immunization series and the first dose of mefloquine or proguanil.

4.5.2 Future vaccines

Vi-rEPA

A new Vi conjugate candidate vaccine bound to non-toxic recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA) has enhanced immunogenicity in adults and in children aged 5–14 years, and has induced a booster response in children aged 2–4 years (81). In a double-blind randomized field trial, 11 091 Vietnamese children aged 2–5 years were given two injections of Vi-rEPA separated by six weeks (82). No serious side-reactions were observed. The efficacy after 27 months of active surveillance was 91.2%. Passive surveillance in the 16 months since the study ended (three-and-a-half years after the first injection) showed 88% efficacy.

S. paratyphi A causes the second commonest enteric fever in Asia. The TAB vaccine, composed of inactivated *Salmonella*, caused a strong side-reaction. A new *S. paratyphi* A vaccine composed of the surface O-specific polysaccharide conjugated with tetanus toxoid was shown to be safe and immunogenic in Vietnamese adults, 108 teenagers and 110 children aged 2–4 years (83). An efficacy trial is being planned.

Other candidates

Three live attenuated candidate vaccines are currently being evaluated. Each is administered as a single oral dose. CVD 908-htrA is an *S. typhi* strain with a mutation deletion in the *htrA* gene (84, 85); a derivative strain, CVD 909, was prepared in order to produce Vi antigen according to constitutive expression. The second candidate is an *S. typhi* Ty2 strain with triple mutation deletion in the cya, crp and cdt genes (86). The third is a derivative of an *S. typhi* Ty2 strain with a double mutation deletion in genes *phoP* and *pho*Q (87).

4.5.3 Recommendations on vaccine use

The occurrence of *S. typhi* strains that are resistant to fluoroquinolones emphasizes the need to use safe and effective vaccines to prevent typhoid fever. WHO recommends vaccination for people travelling in high-risk areas where the disease is endemic. People living in such areas, people in refugee camps, microbiologists, sewage workers and children should be the target groups for vaccination.

Routine immunization

During the 1980s, typhoid fever was successfully controlled in Bangkok by annual routine immunization of school-age children (88). The disease reappeared few years after immunization was stopped. Routine immunization is conducted in several areas of Uzbekistan, resulting in a low incidence of the disease. **WHO recommends that the immunization of school-age children be undertaken wherever the control of the disease is a priority**. School-based typhoid immunization programmes should be limited to geographical areas where typhoid fever is a recognized public health problem and to areas where antibiotic-resistant *S. typhi* strains are particularly prevalent. The use of typhoid vaccines in schoolchildren should be harmonized with the school-based administration of Td (see *Report of the Scientific Group of Experts (SAGE)*, WHO/GPV/98.06, and *Strategies, policies and practices for immunization of adolescents: a global review*, WHO, 1999). The Vi vaccine is recommended for use in immunocompromised hosts. Because some countries, e.g. Bangladesh and India, are reporting typhoid fever cases among the very young, immunization should be started in nursery school children. **In routine immunization, therefore, the use of the available typhoid vaccines should be considered in areas where typhoid fever is endemic in children aged over two years. Either Vi or Ty21a vaccine should be used.**

Immunization in outbreak situations

During 1998 in Tajikistan the vaccination of 18 000 persons with one i.m. dose of Vi polysaccharide proved effective (72% protection) in preventing the spread of typhoid fever in an immunized community facing an outbreak situation because of the presence of a multidrug-resistant strain of *S. typhi* (89). In China's Xing-An county (78) a locally produced Vi vaccine provided 70% protection in school-age children immunized either before or during an outbreak. **Vaccination against typhoid fever before or during an outbreak situation should therefore be seriously considered as an effective tool**. If the community in question cannot be fully immunized, **persons aged 2–19 years should be the target group** for vaccination, in addition to children in nursery schools.

Conclusions

Infection caused by *S. typhi* remains an important public health problem, particularly in developing countries. Morbidity and mortality attributable to typhoid fever are once again increasing with the emergence and worldwide spread of *S. typhi* strains that are resistant to most previously useful antibiotics. As a consequence there is renewed interest in understanding the epidemiology, diagnosis and treatment of typhoid fever and some specific aspects of its pathogenesis. More importantly, perhaps, there is much interest in the possibility of expanded roles for typhoid vaccines. Public health authorities should now devise ways of using the two currently available improved typhoid vaccines, parenteral Vi polysaccharide and oral Ty21a, in large-scale nursery-based and school-based immunization programmes, and should monitor their public health impact.

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The Department of Vaccines and Biologicals was established by the World Health Organization in 1998 to operate within the Cluster of Health Technologies and Pharmaceuticals. The Department's major goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases.

Five groups implement its strategy, which starts with the establishment and maintenance of norms and standards, focusing on major vaccine and technology issues, and ends with implementation and guidance for immunization services. The work of the groups is outlined below.

The *Quality Assurance and Safety of Biologicals team* team ensures the quality and safety of vaccines and other biological medicines through the development and establishment of global norms and standards.

The *Initiative for Vaccine Research* and its three teams involved in viral, bacterial and parasitic diseases coordinate and facilitate research and development of new vaccines and immunization-related technologies.

The *Vaccine Assessment and Monitoring team* assesses strategies and activities for reducing morbidity and mortality caused by vaccine-preventable diseases.

The *Access to Technologies team* endeavours to reduce financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies.

The *Expanded Programme on Immunization* develops policies and strategies for maximizing the use of vaccines of public health importance and their delivery. It supports the WHO regions and countries in acquiring the skills, competence and infrastructure needed for implementing these policies and strategies and for achieving disease control and/or elimination and eradication objectives.

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