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Shigellosis

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Shigellosis is a global human health problem. Four species of *Shigella* i.e. *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei* are able to cause the disease. These species are subdivided into serotypes on the basis of O-specific polysaccharide of the LPS. *Shigella dysenteriae* type 1 produces severe disease and may be associated with life-threatening complications. The symptoms of shigellosis include diarrhoea and/or dysentery with frequent mucoid bloody stools, abdominal cramps and tenesmus. *Shigella* spp. cause dysentery by invading the colonic mucosa. *Shigella* bacteria multiply within colonic epithelial cells, cause cell death and spread laterally to infect and kill adjacent epithelial cells, causing mucosal ulceration, inflammation and bleeding. Transmission usually occurs via contaminated food and water or through person-to-person contact. Laboratory diagnosis is made by culturing the stool samples using selective/differential agar media. *Shigella* spp. are highly fragile organism and considerable care must be exercised in collecting faecal specimens, transporting them to the laboratories and in using appropriate media for isolation. Antimicrobial agents are the mainstay of therapy of all cases of shigellosis. Due to the global emergence of drug resistance, the choice of antimicrobial agents for treating shigellosis is limited. Although single dose of norfloxacin and ciprofloxacin has been shown to be effective, they are currently less effective against *S. dysenteriae* type 1 infection. Newer quinolones, cephalosporin derivatives, and azithromycin are the drug of choice. However, fluoroquinolone-resistant *S. dysenteriae* type 1 infection have been reported. Currently, no vaccines against *Shigella* infection exist. Both live and subunit parenteral vaccine candidates are under development. Because immunity to *Shigella* is serotype-specific, the priority is to develop vaccine against *S. dysenteriae* type 1 and *S. flexneri* type 2a. *Shigella* species are important pathogens responsible for diarrhoeal diseases and dysentery occurring all over the world. The morbidity and mortality due to shigellosis are especially high among children in developing countries. A recent review of literature (Kotloff et al.,1999) concluded that, of the estimated 165 million cases of *Shigella* diarrhoea that occur annually, 99% occur in developing countries, and in developing countries 69% of episodes occur in children under five years of age. Moreover, of the ca.1.1 million deaths attributed to *Shigella* infections in developing countries, 60% of deaths occur in the under-five age group. Travellers from developed to developing regions and soldiers serving under field conditions are also at an increased risk to develop shigellosis.

Key words: Shigellosis, Serotypes, Dysentery, Pathogenesis, Laboratory diagnosis, Treatment, Drug resistance, Vaccine

History of discovery of Shiga bacillus

S. dysenteriae type 1, the first *Shigella* species isolated, was discovered by Kiyoshi Shiga in 1896 (Shiga, 1898). He was born as the fifth child of Shin and Chiyo Sato on 5th February 1871 in Sendai, in northern Japan. His early years were difficult and due to economic hardship young Kiyoshi was raised in his maternal family and later adopted his mothers maiden name, Shiga, as his surname. In 1886 his family moved to Tokyo, where Shiga attended high school. He entered the Tokyo Imperial University School of Medicine in 1892 (Trofa, 1999) During medical school young Shiga was much impressed by Dr. Shibasaburo Kitasato, who achieved international recognition in 1889 with his successful pure cultivation of *Clostridium tetani* and his discovery of tetanus-antitoxin, with the promise of immunotherapy (Behring, 1890). In 1894 Kitasato had investigated a bubonic plague epidemic in Hong Kong and reported his findings in the *Lancet* (Kitasato, 1894). After graduation Shiga entered the Institute for Infectious Diseases, established and directed by Kitasato, as a research assistant. Shiga was initially assigned to the tuberculosis and diphtheria wards, but in late 1897 Kitasato directed his attention to the microbiological investigation of a *sekiri* (dysentery) outbreak. The meaning of the Japanese word *sekiri*, derived from Chinese characters that indicated "red diarrhoea". Dysentery epidemics, affecting tens of thousands with high mortality occurred

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periodically in Japan during the last decade of the 19th century (Shiga, 1906). The 1897 *sekiri* epidemic affected > 91,000 with a mortality rate of > 20%. Shiga studied 36 dysentery patients at the Institute of Infectious Diseases. He isolated a bacillus from stool that was negative by gram-staining, fermented dextrose, was negative in the indole reaction and did not form acid from mannitol. Subculture of the organism caused diarrhoea when fed to dogs. The key to his remarkable discovery, however, was a simple agglutination technique. Shiga demonstrated that the organism repeatedly coalesced when exposed to the serum of convalescent dysentery patients. He published his findings with a gracious acknowledgment of Dr. Kitasato's guidance (Shiga, 1898).

Shiga continued to characterize the organism, initially termed *Bacillus* dysenterie (Shiga, 1906). In particular, he described the production of toxic factors by the organism. One of these factors, now known as Shiga toxin, was recently reviewed in a historical context (Keusch, 1998). In the years immediately following Shiga's discovery of the dysentery bacillus, similar organisms were reported by other investigators (Flexner, 1900; Kruse, 1900) and over the next 40 years three additional groups of related organisms were defined ultimately and taxonomically placed in the genus *Shigella* and named *S. dysenteriae, S. flexneri, S. boydii, and S. sonnei* to honour the lead workers, Shiga, Flexner, Boyd, and Sonne (Hale, 1991). Several revisions in the nomenclature followed. The genus was first termed *Shigella* in the1930 edition of *Bergey's Manual of Determinative Bacteriology* (Bergey's Manual, 1930).

Dr. Shiga died at the age of 85 years on 25 January 1957. His obituary in the *New York Times* stated that he could be considered as one of the four or five most eminent men in bacteriology in his most active years (Obituary, 1957)

The pathogen

Organisms of the genus *Shigella* belong to the tribe *Escherichia* in the family Enterobacteriaceae. It is a small, uncapsulated, non-motile Gram negative nonsporulating, facultative anaerobic bacilli. In DNA hybridization studies, *Escherichia coli* and *Shigella* species cannot be differentiated on the polynucleotide level; however, the virulence phenotype of the later species is a distinctive distinguishing feature. Enteroinvasive *E. coli* (EIEC) are very similar to Shigellae biochemically and they also evoke diarrhoea and/or dysentery. Some EIEC are also serologically related to Shigellae. For example, EIEC serotype 124 agglutinates in *S. dysenteriae* type 3 antiserum. There are four species of *Shigella* classified on the basis of biochemical and serological differences: *S. dysenteriae* (serogroup A, consisting of 13 serotypes); *S. flexneri* (serogroup B, consisting of 15 serotypes [including subtypes]); *S. boydii* (serogroup C, consisting of 18 serotypes); and *S. sonnei* (serogroup D, consisting of a single serotype). This is based on the O antigen component of lipopolysaccharide present on the outer membrane of the cell wall. Serogroups A, B, and C are very similar physiologically while *S. sonnei* can be differentiated from the other serogroups by positive beta-D-galactosidase and ornithine decarboxylase biochemical reactions. *S. dysenteriae serotype 1,* also known as the Shiga bacillus has been recognized as the major cause of epidemic dysentery. During the past 40 years pandemic of Shiga dysentery have spread across worldwide (Gangarosa *et al,* 1970; Mata *et al,* 1970; Rahaman *et al* 1975; Pal, 1984).

Epidemiology

Shigellosis is a global human health problem. Today, more than 100 years after Shiga's landmark discovery, shigellosis is still an important public health problem, especially in developing countries, with substandard hygiene and unsafe water supplies. Humans are the only natural hosts for *Shigella*. Worldwide, the incidence of shigellosis is highest among children 1 to 4 years of age, but during *S. dysenteriae* type 1 epidemics all age groups are affected. In children who are malnourished, *Shigella* often causes a vicious cycle of further impaired nutrition, recurrent infection, and further growth retardation. In the United States and Europe, children in day-care centers, migrant workers, travelers to developing countries, individuals in custodial institutions, and homosexual men are infected most often. The predominant mode of transmission is by faecal-oral contact. Low infectious inoculum (as few as 10 organisms) (DuPont, 1989) renders Shigellae highly contagious. Persons symptomatic with diarrhoea are primarily responsible for transmission. Less commonly, transmission is related to contaminated food and water or fomites; however, the organism generally survives poorly in the environment. In certain settings where disposal of human faeces is inadequqte, flies, particularly *Musca domestica*, the common housefly, may serve as vectors for transmission of shigellosis (Levine, 1991).

No groups of individuals are immune to shigellosis, but certain individuals are at increased risk. Various surveys carried out in treatment centers show that *Shigella* is associated with 5% to 15% of cases of diarrhoea and 30% to 50% of cases of dysentery. Although epidemic Shiga dysentery is the most dramatic manifestation of *Shigella* infection in developing countries, the majority of *Shigella* infections are due to endemic shigellosis. Endemic *Shigella* is responsible for approximately 10% of all diarrhoeal episodes among children younger than five years living in developing countries (Ferreccio *et al.,* 1991) and up to 75% of diarrhoeal deaths (Bennish , 1991; Kotloff *et al.,* 1999). *S. flexneri* is the hyperendemic species in developing countries and is responsible for approximately 10% of all diarrhoeal episode among children younger than five years. Epidemic and endemic disease being caused by *S. dysenteriae* type 1, whereas, in developed

countries, sporadic common source outbreaks, predominantly involving *S. sonnei,* are transmitted by uncooked food or contaminated water and is involved in over 75% of the cases annually in the United States. In general, the illness caused by *S. sonnei* is less severe. The fourth species, *S. boydii,* was first found in India and to this day is uncommonly encountered except in the Indian subcontinent. Curiously, although *S. sonnei* is isolated three times more often than *S. flexneri* in the United States, the latter is the most common in male homosexuals (Drusin, 1976). In many regions of the developing world, the HIV epidemic intersects with spread of shigellosis. HIV- associated immunodeficiency leads to more severe clinical manifestations of *Shigella* infection, including persistent or recurrent intestinal disease and bacteremia (Kristjansson *et al.,* 1994; Angulo *et al.,* 1995; Batchelor *et al.,* 1995).

Immunity to Shigella infections

Several lines of evidences indicate that wild type shigella infection confers protective immunity. In endemic areas, the incidence of shigellosis peaks during the first 5 years of life and declines thereafter, suggesting that immunity develops after repeated exposures during childhood (Taylor *et al,* 1986). The incidence of disease declines with the duration of stay in high-risk settings such as military camps(Cohen *et al.* 1992). Of great relevance to vaccine development is the observation that this immunity is serotype specific (e.g., directed to the LPS O antigen of the organism). Antibody response to the somatic antigens of *Shigella* develop early in infection and follow the typical course for anti-LPS antibodies, that is, an IgM response that peaks within weeks and wanes after 1-2 years.

Compelling evidence of serotype-specific natural immunity comes from longitudinal study of a cohort of Chilean children in whom primary *Shigella* infection conferred 76% protective efficacy against reinfection with the same serotype (Ferreccio *et al,* 1991). Moreover, adult volunteers who are experimentally infected with either *S. sonnei* or *S. flexneri* were significantly protected against illness following rechallenge with the homologous strain (64-74% protective efficacy) (Herrington, 1990; Kotloff, 1995). Thus, an individual convalescent from *S. flexneri* 2a infection is protected against reinfection only with the homologous serotype. This has been the basis for resurgence in interest in LPS determinants for immunization, even by the parenteral route (Robbins, 1992).

Virulence factors and pathogenesis of Shigella

Shigella infection is generally limited to the intestinal mucosa. The ability of *Shigella* to invade and colonize the intestinal epithelium is a key determinant of the disease. The pathogenic mechanism of shigellosis is complex, involving a possible enterotoxic and/ or cytotoxic diarrhoeal prodrome, cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium. The underlying physiological insult that initiates this inflammatory cascade is the invasion of *Shigella* into the colonic epithelium and the lamina propria. The resulting colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhoea. The acute inflammatory response by the host to *Shigella* infection, with the accompanying generation of cytokines, contributes to the disease process.

In recent years much has been learned about the sophisticated virulence mechanisms that allow *Shigella* to invade epithelial cells and spread to neighbouring cells (Finlay, 1997).

Cellular invasion and spread of infection is complex and a good example of multiple gene actions. The process can be arbitrarily divided into at least four stages: (1) cell invasion; (2) intracellular multiplication; (3) intra and intercellular spread; and (4) host cell killing (Sansonetti, 1992). The organism can enter both enterocytes and M cells, which are specialized epithelial cells overlying mucosal lymphoid follicles. The infection process involves multiple steps including macropinocytosis, escape into the cytosol followed by multiplication, and passage to the adjacent cells. The Ipa proteins, which mediate macropinocytosis, are encoded on the "virulence plasmid". The *Shigella* virulence gene is a complicated regulatory cascade and is not completely understood (Berlutti *et al,* 1998; Dorman and Porter, 1998). *Shigella* invades epithelial cells by reorganizing the cytoskeleton, starting with the type III secretion system (Sansonetti and Egile, 1998; Nhieu and Sansonetti, 1999), apparently controlled by GTPases (Mounier *et al,* 1999). Other studies (Hong *et al.,* 1998; McCormack *et al.,* 1998; Way and Goldberg, 1998; Way *et al.,* 1998; Schuch and Maurelli, 1999) have identified different, plasmids and chromosomal loci. Interaction with membrane lipoproteins, lack of dependence on nitric oxide in the clearence of *Shigella* and dependence on γ-IFN in resistance also to be important for the virulence and invasiveness of the different strains of *Shigella.*

Toxins

Just two years after Shiga's complete description of *S. dysenteriae* type 1, Flexner found that parenteral injection of killed *Shigella* cultures in mice led to their death and on this basis concluded that disease was caused by "a toxic agent rather than infection per se." (Flexner, 1900). Three years later, Conradi (Conradi, 1903) found that autolysates of cultures of *S. dysenteriae* type 1 caused diarrhoea, limb paralysis, and death within 48-72 h of intravenous injection into young adult rabbits. Because of these findings, the factor was called Shiga neurotoxin, or just Shiga toxin. Todd (Todd, 1904) soon found that *S. flexneri* filtrates, which also caused diarrhoea when injected did not cause paralysis, indicating specific production of neurotoxin by *S. dysenteriae* type 1, which was

ultimately confirmed when the gene was identified. In retrospect, these early investigators were no doubt seeing the combined effects of lipopolysaccharide (LPS) endotoxin and the protein Shiga toxin. *Shigella* strains produce 3 distinct enterotoxins: (a) chromosome encoded *shigella* enterotoxin 1 (SHET1) which is present in all *S. flexneri* 2a (Venkatessan *et al.,* 1991; Yavzori *et al.,* 2002; Niyogi *et al.*, 2004) but rarely found in other *shigella* serotypes (Noriega, 1995), (b) *shigella* enterotoxin 2 (SHET2) which is located on a large plasmid associated with virulence of *shigella* (Nataro, 1995). SHET2 was found in many, but not all, *shigella* of different serotypes and also in enteroinvasive *Escherichia coli* (EIEC) (Nataro, 1995; Vargas, 1999). The soluble toxins, SHET1 and SHET2, show significant enterotoxic activity *in vitro* when tested in rabbit ileal loops and Ussing chambers. Furthurmore, inactivation of these enterotoxins through genetic engineering is used for attenuation of new *shigella* vaccine candidate (Kotloff, 2000), and (c) phage-borne Shiga toxin by *S. dysenteriae.*

Shiga toxin is neurotoxic, cytotoxic, and enterotoxic, encoded by chromosomal genes, with two domain, 1-A and 5-B structure similar to the Shiga-like toxins of enterohaemorrhagic *E. coli* infection (Acheson, 1991).

Enterotoxic effect

Shiga toxin adheres to small intestine receptors and blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen.

Cytotoxic effect

B subunit of Shiga toxin binds host cell glycolipid in large intestine, A1 domain internalized via receptor-mediated endocytosis and cause irreversible inactivation of the 60S ribosomal subunit, thereby inhibiting protein synthesis, causing cell death, microvasculature damage to the intestine, and haemorrhage (blood and faecal leukocytes in stool).

Neurotoxic effect

Fever and abdominal cramping are considered as signs of neurotoxicity.

Shiga toxin is not essential for virulence of *S. dysenteriae* type 1 in primates but contributes to severity of disease manifestations, especially bloody diarrhoea/dysentery (Fontaine, 1988).

The disease

Shigellosis typically evolves through several phases and manifestations of *Shigella* infection vary with the infecting species, the age of the host, the presence of risk factors and the specific immune status of the host. The incubation period is 1 to 4 days, but may be as long as 8 days with *S. dysenteriae* (Levine *et al.,* 1973). Shigellosis, or acute bacillary dysentery, is an invasive infection of the human colon that affects a spectrum of clinical presentations, from short-lasting watery diarrhoea to inflammatory bowel disease. Clinical disease typically begins within 24-48 hr of ingestion of a few hundred to a few thousand organisms with constitutional symptoms such as fever, fatigue, malaise, and anorexia. Watery diarrhoea typically precedes dysentery (DuPont, 1969) and is often the sole clinical manifestation of mild infection (Taylor, 1986). Progression to frank dysentery may occur within hours to days with frequent small volume of bloody, mucoid stools, abdominal cramps and tenesmus. In patients experiencing dysentery, involvement is most severe in the distal colon, and the resulting inflammatory colitis is evidenced in frequent scanty stools reflecting the ileocaecal fluid flow. Patients with severe infection may pass more than 20 dysenteric stools in one day (Mathan, 1991). Dysentery is also characterized by the daily loss of 200-300 ml of serum protein into the faeces. This loss of serum proteins results in depletion of nitrogen stores that exacerbate malnutrition and growth stunting. Depletion of immune factors also increases the risk of concurrent, unrelated infectious disease and contributes to substantial mortality.

Anorexia, which is a prominent finding initially, may persist into convalescense and contribute to the deterioration in the patients nutritional status, which commonly occurs in shigellosis. Large fluid losses and severe dehydration are rare in shigellosis (Butler, 1986). A variety of unusual extraintestinal manifestations may occur. The most common is seizures, which usually occur in the presence of fever without associated encephalopathy (Ashkenazi *et al.,* 1987). Microangiopathic haemolytic anaemia can complicate infection with Shiga toxin-producing organisms, manifesting as the haemolytic uraemic syndrome in children and as thrombotic thrombocytopenic purpura in adults (Koster et al, 1978). Most episodes of shigellosis in otherwise healthy individuals are self-limited and resolve within 5-7 days without sequlae. Acute, life-threatening complications are most often seen in malnourished infants and young children living in developing countries (Bennish, 1991). These include metabolic derangements, such as dehydration, hyponatraemia, and hypoglycaemia (Bennish, 1991), intestinal complications such as toxic megacolon, rectal prolapse, intestinal perforation (Bennish, 1991) and rarely sepsis (Struelens *et al,* 1985). *Shigella* bacteremia has been reported among HIV-infected and other immunocompromised patients (Kristjansso *et al,* 1994; Batchelor *et al,* 1996). Persistent diarrhoea and malnutrition are the most common chronic sequelae (Black, 1982). A rare post-infectious complication seen primarily in adults following infection with *S. flexneri* serotypes is reactive inflammatory arthritis, alone (Sieper *et al,* 1993) or as part of a constellation of arthritis, conjunctivitis, and urethritis known as Reiter's syndrome (Finch *et al,* 1986). Realistic approaches to the

reduction of mortality from shigellosis must continue to focus on prevention and early antimicrobial therapy rather than on treatment of established complications.

Diagnosis

Clinical

Patients presenting with watery diarrhoea and fever should be suspected of having shigellosis. The diarrhoeal stage of the infection cannot be distinguished clinically from other bacterial, viral, and protozoan infections. Nausea and vomiting may accompany with shigella diarrhoea, but these symptoms are also observed during infections with nontyphoidal Salmonellae and enterotoxigenic *E. coli*. Bloody, mucoid stools are highly indicative of shigellosis, but the differential diagnosis should include infection by EIEC, *Salmonella enteritidis*, *Yersinia enterocolitica*, *Campylobacter* species, and *Entamoeba histolytica*. Although blood is common in stools of patient with amoebiasis, it is usually dark brown rather than bright red, as in shigella infections. Sigmoidoscopic examination of a shigellosis patient reveals a diffusely erythematous mucosal surface with small ulcers, whereas amoebiasis is characterized by discreate ulcers in the absence of generalized inflammation.

Laboratory

Although clinical signs may evoke the suspicion of shigellosis, diagnosis is dependent upon the isolation and identification of shigellae from the faeces. Shigellae remain viable for a limited time outside the human body; therefore, stool specimens should be processed within a few hours after collection (Levine, 1991; Shears, 1996). Faecal specimens should be collected in the early stages of the disease when pathogens usually are present in the stool in high numbers, and preferably before antibiotic treatment is begun. Positive cultures are most often obtained from blood-tinged plugs of mucus in freshly passed stool specimens obtained during the acute phase of disease. Rectal swabs may also be used to culture shigellae if the specimen is processed rapidly or the swab may be placed in Cary-Blair transport medium for preservation and transport of specimen. Buffered glycerol saline (BGS) medium is also much useful for transporting specimens for shigellae. BGS, while still alkaline as indicated by the pink color that persists after the addition of faeces, is considered to be better than Cary-Blair medium. Isolation of shigellae in the microbiological laboratory typically involves an initial streaking for isolation on differential/selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora. Commonly used primary isolation media include MacConkey, Hektoen Enteric, Salmonella-Shigella, Xylose Lysine Desoxycholate and Desoxycholate Citrate agar media. However, *S. dysenteriae type* 1 and *S. sonnei* do not grow well on Salmonella-Shigella agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as shigellae. A liquid enrichment medium (Hajna Gram-negative broth) may also be inoculated with the stool specimen and subcultured onto the selective/differential agar media after a short growth period. Following overnight incubation of primary isolation media at 37°C, colorless, non-lactose fermenting colonies are inoculated into Triple Sugar Iron (TSI) agar slant. In this differential media, *Shigellae* produce an alkaline slant and acid butt with no bubbles of gas in the agar. This reaction gives a presumptive identification, and slide agglutinatin tests with commercially available antisera for serogroup and serotype confirm the identification (WHO/CDD/83.3).

Some *E. coli* biotypes of the normal intestinal flora closely resemble *Shigella* species(i.e, they are non-motile, delayed lactose fermenters). These coliforms can ususlly be differentiated from Shigellae by the ability to decarboxylate lysine. However, some coliforms cause enteroinvasive disease because then may carry the *Shigella*-like virulence plasmid, and these pathogens are conventionally identified by laborious serological screening for EIEC serotypes. Sensitive and rapid techniques for detecting *Shigella* have been developed. These methods utilize gene probes or polymerase chain reaction (PCR) primes directed towards virulence genes such as the invasion plasmid locus (*ipl*) or that encoding the IpaH antigen virulence factor. Although more sensitive than the conventional diagnostic method, these techniques require sophisticated laboratory but they are probably too specializes for routine use in the clinical laboratory.

Antimicrobial susceptibility tests of all the confirmed *Shigella* isolates should be performed using an agar diffusion technique method in accord with the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1997). The agar and broth dilution methods are also widely used (Ackerman and Dello, 1996) Newer methods like epsilometer strip method (E-test) is also a widely used method for accurate determination of minimum inhibitory concentration (MIC) (Olssson-Liljequist, 1992). However, its main drawback is its high cost. Recently, a personal computer-based commercial geographic information system (GIS) was applied to an outbreak of *S. sonnei* infection at Fort Braggy, North Carolina. A GIS offers an efficient and practical way to directly visualize the dynamics of transmission of infectious diseases in the setting of a community outbreak (Mckee KT Jr *et al*, 2000).

Treatment

Rehydration therapy is an essential first step which can be used to correct dehydration due to diarrhoeas of any etiology and has greatly decreased the number of deaths due to diarrhoea. The oral rehydration treatment devel-

oped by the World Health Organization has proven effective and safe, and is an essential component as life-saving therapy for acute dehydrating watery diarrhoea, and the pivotal strategy of global diarrhoeal diseases control program. Although severe dehydration is uncommon in shigellosis, with proper hydration, shigellosis is generally a self-limiting disease, and the decision to prescribe antibiotics is predicted on the severity of the disease, the age of the patient, and the likelihood of further transmission of the infection. An effective oral antimicrobial causes marked symptomatic improvement within 48 hours in shigellosis and reduces the average duration of illness from approximately 5-7 days to approximately 3 days and also reduces the period of *Shigella* excretion after symptoms subside (Salam and Bennish, 1991). Without antimicrobial treatment, or if an ineffective antimicrobial is given, an episode of shigellosis lasts from two to 10 days, or longer, and the risk of serious complications or death is greatly increased, especially for infection caused by *S. dysenteriae* type 1 or *S. flexneri*. Inadequately treated shigellosis is an important cause of persistent diarrhoea

According to the WHO guidelines (WHO/CDR/95.3), when a presumptive diagnosis of shigellosis is made, all such patients should be treated with an antibiotic, the choice being decided by the antimicrobial susceptibility pattern of locally circulating shigella strains. If after 2 days of therapy, the patient condition improves, then a full course of 5 days should be given. On the other hand, if the patient does not improve, the antibiotic should be changed. If improvement is seen after 2 days, it should be continued for a total of 5 days. If, even with the second antibiotic, the patient does not show signs of improvement, the diagnosis must be reviewed and stool microscopy, culture and susceptibility testing should be carried out. However, the WHO guidelines for the treatment of shigellosis may be difficult to strictly follow because of the problem of wide-spread drug resistance.

A variety of antimicrobial agents are effective for treatment of shigellosis, although options are becoming limited due to globally emerging drug resistance (Sack RB *et al,* 1996). *Shigella* resistance to sulfonamides, tetracyclines, ampicillin, and TMP-SMX exists worldwide, and these agents are not recommended as empirical therapy.

In the 1990s, quinolone emerged as the preferred agents for treatment of *Shigella.* All available quinolones have excellent in vitro activity, and multiple trials support their clinical efficacy (Gotuzzo *et al,* 1989; Murphy *et al,* 1993). Most authorities now recommend an oral quinolone (ciprofloxacin, levofloxacin or norfloxacin) for proven or suspected shigellosis. One or 2 doses are reasonable for mild-to- moderate illness, whereas 3 to 5 days of therapy should be used for complicated bacillary dysentery or proven *S. dysenteriae* type 1 infections. Although single dose of norfloxacin 800 mg and ciprofloxacin 1 g have been shown to be effective, they are currently less effective against *S. dysenteriae* type 1 infection (Bhattacharya, 2003). None of the newer fluoroquinolones is approved for use in children or pregnant woman. The use of fluoroquinolone in children has been limited because these drugs have the potential of inducing cartilage toxicity. However, there is growing evidence that they will prove safe (Bhattacharya SK, 1997; Gendrel *et al,* 2001). Various antibiotics as well as the optimal duration of therapy have been carefully evaluated. Most controlled studies have used 5 days of treatment, also shorter course of therapy, have also been explored (Salam and Bennish, 1991).

Given that quinolone resistance is increasing and that there are concerns about their safety in children, the search for other agents continues. First-generation and second-generation cephalosporins are active in vitro but disappointing in clinical use. Studies using cefixime, an oral third-generation cephalosporin, in adults with shigellosis were unimpressive with only a 53% success rate (Salam MA *et al*, 1995). However, clinical trial in Israel demonstrated that cefixime and ceftriaxone, have better rates of bacteriological and clinical cure and that they are safe for use in children (Varsano *et al.,* 1991; Ashkenazi *et al.,* 1993). Recently, azithromycin., a macrolide with excellent intracellular penetration and modest in vitro *Shigella* activity, has been shown to be effective in the treatment of shigellosis (Khan, 1997; Basualdo, 2003). Other options that need further testing is a non-absorbed antimicrobial, rifaximin (DuPont *et al.,* 1998) There is not a complete correlation between *in vitro* antibiotic susceptibility and clinical efficacy. Although the infecting organism must be sensitive to the antibiotic being used, several antibiotics that active *in vitro* have been ineffective clinically. Using an ineffective antibiotic, that is one to which the organism is resistant or that is clinically ineffective, may pose a risk. In addition to any potential systemic side-effects of the drug, it may affect the normal intestinal flora. There is evidence that the normal flora compete with the infecting shigellae; thus, an ineffective antibiotic may actually exacerbate the disease by selectively promoting the shigellae. (WHO/CDS/CSR/DRS/2001. 8.). Moreover, the shift in the prevalence of serogroups and the changing pattern in antimicrobial susceptibilities among *Shigella* isolates poses a major difficulty in the determination of an appropiate drug for the treatment of shigellosis. Continuous monitoring of antimicrobial susceptibilities of *Shigella* spp. through a surveillance system is thus essential for effective therapy and control measures against shigellosis (Niyogi, 2001).

Complications such as seizures, encephalopathy, and intestinal perforation require specific therapy in addition to antimicrobials and fluids

Antimicrobial resistance

According to the history of this genus, *Shigella* spp. can easily become resistant to antibiotics (WHO 2001). Over

the past several decades, shigella strains have progressively become resistant to most of the widely used and inexpensive antimicrobials resulting in treatment failure and increased mortality (Levine MM, 1986).

Multi-drug resistance is a serious problem for the treatment of shigellosis, particularly those caused by *S. dysenteriae* type 1. Sulfonamides, which were highly effective in the 1940s, had little practical value by the 1950s. Later in the 1960s, tetracycline, followed by ampicillin and co-trimoxazole, were found to be highly beneficial (Nelsen JD, 1976). Resistance to these three drugs became high in the late 1960s through to the 1980s (Ross, 1972). In the early 1980s, nalidixic acid, a first generation quinolone derivative, which was effective initially and highly encouraging results with the use of this drug in the treatment of multi-resistant *S. dysenteriae* type 1 infection were reported from Calcutta (Bose, 1984). Subsequently, studies showed that nalidixic acid is highly effective in the treatment of shigellosis in children and adults (Bhattacharya S K, 1987; Salam and Bennish, 1988). However, within a short period the widespread use of the drug resulted in the emergence of nalidixic-acid resistant *S. dysenteriae* type 1 strains in several parts of the world (Panhotra *et al*, 1985; Munshi *et al*, 1987; Sen *et al*, 1988). Later it was documented that newer fluoroquinolones, such as norfloxacin and ciprofloxacin, appeared to be superior to nalidixic acid in the treatment of shigellosis caused by nalidixic acid-resistant *Shigella* strains (Bennish *et al*, 1990; Bhattacharya *et al*, 1991; Bhattacharya *et al*, 1992). These antibiotics are more expensive, and recently emergence of resistance has already developed due to their unrestricted and widespread use. The antimicrobials that remain effective are mecillinam, ciprofloxacin and other fluoroquinolones, ceftriaxone and azithromycin. However, in 2003 a few cases of ciprofloxacin- and other fluoroquinolones-resistant *S. dysenteriae* type 1 infection have been reported from India, Bangladesh and Nepal (Niyogi, 2001; Bhattacharya and Sur, 2003; Sarkar *et al*, 2003; Sur *et al*, 2003). There are also geographic differences in the resistance rates that can be quite striking. The antimicrobial resistance pattern differs from place to place even in the same place in two separate regions. This may be due to the occurrence and spread of antimicrobial-resistant clones.

Control strategies

As is the case with other enteric infections, the most effective methods for controlling shigellosis are provision of safe and abundant water and effective faeces disposal. These public health measures are, at best, long range strategies for control of enteric infections in developing countries. The most effective intervention strategy to minimize morbidity and mortality would involve comprehensive media and personal outreach programs consisting of the following components:

1. Education of all residents to actively avoid faecal contamination of food and water and to encourage hand washing after daefecation;
2. Encourage mother to breast feed infants;
3. Promote the use of oral rehydration therapy to offset the effects of acute diarrhoea;
4. Encourage mothers to provide convalescent nutritional care in the form of extra food for children recovering from diarrhoea or dysentery.

Vaccines

Although, *S. dysenteriae* type 1 was discovered as the cause of epidemic dysentery in Japan in 1898, there is neither a licensed vaccine for it nor a consensus as to the mechanism(s) of host immunity to *Shigella*. Vaccine development has been hampered by three factors: (i) the ineffectiveness of parenterally injected inactivated whole-cell vaccines which led to the belief that serum antibodies do not confer immunity (Formal SB 1967); (ii) the lack of a suitable animal model (Robbins, 1992); (iii) only indirect evidence of immune mechanism(s) in humans (Robbins 1992,1995; Cohen *et al*,1997)

In addition to work on the pathogenesis of bacillary dysentery, Kiyoshi Shiga focused his efforts on the development of a Shigella vaccine. In his autobiography he describes how he initially prepared a heat-killed whole-cell vaccine and injected himself as the first study subject. The resulting local reaction was severe and required incision and drainage (Shiga K, 1950). He then developed a serum-based passive immunization and later an oral vaccine, which was administered to thousands of Japanese citizens. These experiment were conducted before the advent of controlled clinical trials, and his observations were published primarily in German- and Japanese- language journals. Shiga later expressed reservations about the efficacy of vaccines for the control of enteric diseases and emphasized the importance of public health practices (Shiga, 1936).

To date, no vaccine has been both safe and effective, starting with parenteral heat- or acetone-killed whole-cell vaccines, which induced circulating antibody but little or no protection (Hale, 1992). Contemporary vaccine development has therefore concentrated on use of live attenuated vaccine strains. Advances in biotechnology and considerable advances in our understanding of the molecular mechanism of virulence of *Shigella* have enabled the development of a new generation of candidate vaccines. The state of progress in the development and testing of *Shigella* vaccines was recently reviewed at a meeting convened by the World Health Organization (WHO weekly Epid Rec, 1997).

Recent *Shigella* vaccine candidates have been based on attenuated *S. flexneri* or *S. sonnei* strains, killed *S. flexneri* strains or specific synthetic polysaccharides, and have been shown to be safe and immunogenic in animal mo-

dels (Guan and Verma, 1998; Hartman and Venkatesan, 1998; Noriega *et al.*, 1999; Chakrabarti *et al* 1999; Coster *et al.*, 1999, Pozsgay *et al* 1999). A vaccine composed of specific polysaccharide conjugates of *S. flexneri* and *S. sonnei* has demonstrated safety and immunogenecity in children (Ashkenazi *et al*, 1999). Some of these vaccines have entered clinical trials and show great promise for the prevention of *Shigella* disease (Sansonetti, 1989; Noriega, 1996; Cohen, 1997).

Because immunity in *Shigella* is serotype-specific, the protective performance of an anti-Shigella vaccine in any particular setting will depend in part on the representation of serotypes in the vaccine and on the relative epidemiological importance of different serotypes in that setting. Thus knowledge of the distribution of serotypes among clinical isolates is of crucial importance in designing new vaccines and in judging their suitability for use in public health programs. The priority is to develop vaccines against S. *dysenteriae* type 1 and *S. flexneri* type 2a. Shigellosis, which continues to have an important global impact, cannot be adequately controlled with the existing prevention and treatment measures. Innovative strategies, including development of vaccine against the most common serotypes, could provide substantial benefit.

Future issues

Although several hospital based studies document the relative importance of shigellosis, there have been few studies with a defined population denominator that allowed calculation of incidence rate in the community. There is a need to establish the incidence, prevalence, disease burden and serotype distribution of shigellosis in many areas of the world so that country, regional and global estimates can be made. New inexpensive antimicrobials that are safe as treatment for children are clearly needed.

There is little information on the rate with which infected persons seek outpatient and inpatient medical care, or other similar measures of disease severity.

It is well documented that Shigella spp. are fragile organisms that may be missed in routine microbiological evaluations of faecal specimens. Considerable care must be exercised in collecting faecal specimens, transporting them to the laboratories, and in using appropriate media for isolation.

Antimicrobial resistance constitutes an important element. Patterns of antibiotic resistance, which vary considerably from place to place and which are in a continuous state of evolution, must also be updated.

Estimates of case-fatality are needed from different regions in the developing world. Targeted interventions which have proved effective in reducing *Shigella* infection such as hand-washing programs, encouraging breast feeding of infants and small children, latrine programs to reduce environmental contamination and programs to reduce the density of flies which can deposit infectious inocula of *Shigella* on food should be strengthen.

All the different vaccines approaches developed so far should continue to be evaluated. An ideal vaccine should be easy to administer, preferably orally, although parenteral vaccines should not be discarded if all the following requirements are met; well tolerated; able to induce a high-level long-term protection after a single dose; multivalent; directed against the most representative *Shigella* serotypes causing endemic and epidemic infections; and easy to manufacture.

References

Acheson, D.W.K., A. Donohue-Rolfe, and G.T. Keusch.1991. The family of Shiga and Shiga-like toxins. p. 415-433. *In* J E Alouf and J H Freer (ed.), Sourcebook of Bacterial Protein Toxins. Academic Press, New York.

Ackerman, B.H and F.A Dello Buono. 1996. In vitro testing of antibiotics. *Pharmacotherapy* 16, 201-221.

Angulo, F.J. and D.L. Swerdlow. 1995. Bacterial enteric infections in persons infected with human immunodeficiency virus. *Clin. Inf. Dis.* 21 Suppl. 1, S84-S93.

Ashkenazi, S., G. Dinari, A. Zevulunov, and M. Nitzan. 1987. Convulsions in childhood shigellosis. Clinical and laboratory features in 153 children. *Am. J. Dis. Child.* 141, 208-210.

Ashkenazi, S, J. Amir, Y. Waisman, A. Rachmel, B.Z. Garty, Z. Samra, I. varsano, and M.M. Nitzan. 1993. A randomized, double-blind study compairing cefixime and trimethoprim-sulphamethoxazole in the treatment of childhood shigellosis. *J. Paediatr.* 123, 817-821.

Ashkenazi, S., J.H. Passwell, E. Harlev, D. Miron, R. Dagan, N. Farzan, R. Ramon, F. Majady, D.A. Bryla, A.B. Karpas, J.B. Robbins, and R. Schneerson. The Israel Paediatric *Shigella* Study Group. 1999. Safety and immunogenecity of *Shigella sonnei* and *Shigella flexneri* 2a O specific polysaccharide conjugates in children. *J. Infect. Dis.* 179, 1565-1568.

Basualdo, W., and A. Arbo. 2003. Randomized comparison of azithromycin versus cefixime for treatment of shigellosis in children. *Pediatr. Infect. Dis. J.* 22(4), 347-377.

Batchelor, B.I, J.N. Kimari, and R.J. Brindle. 1996. Microbiology of HIV associated bacteraemia and diarrhoea in adults from Nairobi, Kenya. *Epidemiol. Infect.* 117,139-144.

Behring. E. and S. Kitasato. 1890. Ueber das Zustandkommen der Diphtherie-Immunitaet und der Tetanus-Immunitaet bei Thieren. *Dtsch. Med. Wochenschr.* 49,1-6

Bennish, M.L., MA. Salam, R. Haider, and M. Barza.1990. Therapy for Shigellosis. II. Randomized,double-blind comparison of ciprofloxacin and ampicillin. *J. Infect. Dis.* 162, 711-716.

Bennish, M.L. 1991. Potentially lethal complications of shigellosis. *Rev. Infect. Dis.* 13(Suppl. 4), S319-324.

Bennish, M.L., and B.J. Wojtyniak. 1991. Mortality due to shigellosis: community and hospital data. *Rev. Infect. Dis.* 13(Suppl. 4), S245-251.

Bergey's manual of Determinative Bacteriology. 3rd ed. Williams & Wilkins Co., Baltimore, Maryland 1930: 357-363.

Berlutti, F., M. Casalino, C. Zagaglia, P.A. Frandiani, P. Visca, and M. Nicoletti. 1998. Expression of the virulence plasmid-carried apyrase gene (*apy*) of enteroinvasive *Escherichia coli* and *Shi-*

gella flexneri is under the control of H-NS and the VirF and VirB regulatory cascade. *Infect. Immun.* 66, 4957-4964.

Bhattacharya, M.K., G.B. Nair, D. Sen, M. Paul, A. Debnath, A. Nag, D. Dutta, P. Dutta, S.C. Pal, and S.K. Bhattacharya. 1992. Efficacy of norfloxacin for shigellosis: A double-blind randomized clinical trial. *J. Diarrhoeal. Dis. Res.* 10, 146-150.

Bhattacharya, S.K., P. Dutta, D. Dutta, M.K. Bhattacharya, D. Sen, M.R. Saha, G.B. Nair, P. Das, S.N. Sikdar, R. Bose, and S.C. Pal. 1987. Relative efficacy of trimethoprim-sulfamethoxazole and nalidixic acid for acute invasive diarrhoea. *Antimicrob. Agent Chemother.* 31, 837.

Bhattacharya, S.K., M.K. Bhattacharya, P. Dutta, D. Sen, R. Rasaily, A. Moitra, and S.C. Pal. 1991. Randomized clinical trial of norfloxacin for shigellosis. *Am. J. Trop. Med. Hyg.* 45, 683-687.

Bhattacharya, S.K., M.K. Bhattacharya, D. Dutta, S. Dutta, M. Deb, A. Deb, K.P. Das, H. Koley, and G.B. Nair. 1997. Double blind, randomized clinical trial for safety and efficacy of norfloxacin for shigellosis in children. *Acta Paediatr.* 86, 319-320.

Bhattacharya, S.K., and D. Sur. 2003. An evaluation of current shigellosis treatment. *Expert Opin. Pharmacothe.* 4(8), 1315-1320.

Bhattacharya, S.K., K. Sarkar, G.B. Nair, A.S.G. Faruque, and D.A. Sack. 2003 A regional alert of multidrug-resistant *Shigella dysenteriae* type 1 in South Asia. *The Lancet. Inf. Dis.* 3, 751.

Black, R.E., K.H. Brown, S. Becker, A.R. Alim, and I. Huq. 1982. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. II. Incidence of diarrhoea and association with known pathogen. *Am. J. Epidemiol.* 115, 315-324.

Bose, R., J.N. Nashipuri, P.K. Sen, P. Dutta, S.K. Bhattacharya, D. Dutta, D. Sen, and M.K. Bhattacharya. 1984. Epidemic of dysentery in West Bengal: clinicians enigma. *Lancet.* 2, 1160.

Butler, T., P. Speelman, I. Kabir, and J. Banwell. 1986. Colonic dysfunction during shigellosis. *J. Infect. Dis.* 154, 817-824.

Chakrabarti, M.K., J. Bhattacharya, M.K. Bhattacharya, G.B. Nair, S.K. Bhattacharya, and D. Mahalanabis. 1999. Killed oral *Shigella* vaccine made from *Shigella flexneri* 2a protects against challenge in the rabbit model of shigellosis. *Acta Paediatr.* 88, 161-165.

Cohen, D., M.S. Green, C. Block, R. Slepon, and Y. Lerman. 1992. Natural immunity to shigellosis in two groups with different previous risks of exposure to *Shigella* is only partly expressed by serum antibodies to lipopolysaccharide. *J. Infect. Dis.* 165, 785-787.

Cohen, D., S. Ashkenazi, M.S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, I. Ashkenazi, J. Shemer, D.N. Taylor, T.L. Hale, J.C. Sadoff, D. Pavliakova, R. Schneerson, and J.B. Robbins. 1997. Double-blind vaccine-controlled randomized efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. *Lancet.* 349, 155-159.

Conradi, H. 1903. Uber losliche, durch aseptische autolyse erhaltene giftstoffe von ruhrund typhus bazillen. *Dtsch. Med. Wochenschr.* 20, 26-28.

Coster, T.S., C.W. Hoge, L.L. Van DeVerg, A.B. Hartman, E.V. Oaks, M.M. Venkatesan, D. Cohen, G. Robin, A.F. Thompson, P.J. Sansonetti, and T.L. Hale. 1999. Vaccination against shigellosis with attenuated *Shigella flexneri* 2a strain SC602. *Infect. Immun.* 67, 3437-3443.

Dorman, C.J. and M.E. Porter. 1998. The *Shigella* virulence regulatory cascade: a paradigm bacterial gene control mechanism. *Mol. Microbiol.* 29, 677-684.

Drusin, L.M. G. Genvert, B. Topf-Olstein, and E. Levy-Zombeck. 1976. Shigellosis: Another sexually transmitted disease? *Br. J. Vener. Dis.* 52, 348-350.

DuPont, H.L., R.B. Hornick, A.T. Dawkins, M.J. Snyder, and S.B. Formal. 1969. The response of man to virulent *Shigella flexneri* 2a. *J. Infect. Dis.* 119, 296-299.

Du Pont, H.L., M.M. Levine, R.B. Hornick, and S.B Formal. 1989. Inoculum size in shigellosis and implications for expected mode of transmission *J. Infect. Dis.* 159, 1126-1128.

DuPont, H.L., C.D. Ericsson, J.J. Mathewson, E. Palazzini, M.W. DuPont, Z.D. Jiang, A. Mosavi, and F.J. de la Cabada. 1998. Rifaximin, a nonabsorbed antimicrobial, in the therapy of travelers diarrhoea. *Digestion.* 59, 708-714.

Ferreccio, C., V. Prado, A. Ojeda, M. Cayyazo, P. Abrego, L. Guers, and M.M. Levine. 1991. Epidemiologic patterns of acute diarrhoea and endemic *Shigella* infections in a poor periurban setting in Santiago, Chile. *Am. J. Epidemiol.* 134, 614-627.

Finch, M., G. Rodey, D. Lawrence, and P. Blake. 1986. Epidemic Reiters syndrome following an outbreak of shigellosis. *Eur. J. Epidemiol.* 2, 26-30.

Finlay, B.B. and S.Falkow. 1997. Common themes in microbial pathogenecity revisited. *Microbiol. Mol. Biol. Rev.* 61, 136-169.

Flexner, S. 1900. On the etiology of tropical dysentery. *Bull. Johns Hopkins Hosp.* 11,231-242.

Fontaine, A., J. Arondel, and P.J. Sansonetti. 1988. Role of Shiga toxin in the pathogenesis of bacillary dysentery studied using a *tox* mutant of *Shigella dysenteriae* type 1. *Infect. Immun.* 56, 3099-3109.

Formal, S.B., R.M. Maenza, S. Austin, and E.H. LaBrec. 1967. Failure of parenteral vaccine to protect monkeys against experimental shigellosis. *Proc. Soc. Exp. Biol. Med.* 125, 347-349.

Gangarosa, E.J., D.R. Perera, L.J. Mata, C.M. Morris, G. Guzman, and L.B. Reller. 1970. Epidemic Shiga bacillus dysentery in Central America. II. Epidemiologic studies in 1969. *J. Infect. Dis.* 122, 181-190.

Gendrel, D. and F. Moulin. 2001. Fluoroquinolones in paediatrics. *Paediatr. Drugs.* 3(5), 365-377.

Gotuzzo, E., R.A. Oberhelman, C. Maguina, S.J. Berry, A. Yi, M. Guzman, R. Ruiz, R. Leon-Barua, and B. Sack. 1989. Comparison of single-dose treatment with norfloxacin and standard 5-day treatment with trimethoprim-sulphamethoxazole for acute shigellosis in adults. *Antimicrob. Agents Chemother.* 33, 1101-1104.

Guan, S. and N.K. Verma. 1998. Serotype conversion of a *Shigella flexneri* candidate vaccine strain via a novel site-specific chromosome-intigration system. *FEMS Microbiol. Lett.* 166, 79-87.

Hartman, A.B. and M.M. Venkatesan. 1998. Construction of a stable attenuated *Shigella sonnei* Delta virG vaccine strain, WRSS1, and protective efficacy and immunogenecity in the guinea pig keratoconjunctivitis model. *Infect. Immun.* 66, 4572-4576.

Hale, T.L. 1991. Genetic basis of virulence in *Shigella* species. *Microbiol. Rev.* 55, 206-224.

Herrington, D.A., L. Van DeVerg, S.B. Formal, T.L. Hale, B.D. Tall, S.J. Cryz, E.C. Tramont, and M.M. Levine. 1990. Studies in volunteers to evaluate candidate *Shigella* vaccine: further experience with a bivalent *Salmonella typhi-Shigella sonnei* vaccine and protection conferred by previous *Shigella sonnei*

disease. *Vaccine* 8, 353-357.

Hong, M., M. Gleason, E.E. Wyckoff, and S.M. Payne. 1998. Identification of two *Shigella flexneri* chromosomal loci involved in intercellular spreading. *Infect. Immun.* 66, 4700-4710.

Keusch, G.T. 1998. The rediscovery of Shiga toxin and its role in clinical disease. *Jpn. J. Med. Sci. Biol.* 51(Suppl. I), S5-S22.

Khan, W.A., C. Seas, U. Dhar, M.A. Salam, and M.L. Bennish. 1997. Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. A double-blind randomized controlled trial. *Ann. Intern. Med.* 126(9), 697-703.

Kitasato, S. 1894. The bacillus of bubonic plague. *Lancet.* 2, 428-430.

Koster, F., J. Levin, L. Walker, K.S. Tung, R.H. Gilman, M.M. Rahaman, M.M. Majid, S. Islam, and R.C. Williams. 1978. Hemolytic-uremic syndrome after shigellosis. Relation to endotoxemia and circulating immune complexes. *N. Eng. J. Med.* 298, 927-933.

Kotloff, K.L., J.P. Nataro, G.A. Losonsky, S.S. Wasserman, T.L. Hale, D.N. Taylor, J.C. Sadoff, and M.M. Levine. 1995. A modified *Shigella* volunteer challenge model in which the inoculum is administered with bicarbonate buffer: clinical experience and implications for *Shigella* infectivity. *Vaccine.* 13, 1488-1494.

Kotloff, K.L., J.P. Winickoff, B. Ivanoff, J.D. Clemens, D.L. Swerdlow, P.J. Sansonetti, G.K. Adak, and M.M. Levine. 1999. Global burden of *Shigella* infection:Implications for vaccine development and implementation of control strategies. *Bull. WHO.* 77, 651-666.

Kotloff, K.L., F.R. Noriega, T. Samandari, M.B. Sztein, G.A. Losonsky, J.P. Nataro, W.D. Picking, E.M. Barry, and M.M. Levine. 2000. *Shigella flexneri* 2a strain CVD 1207, with specific deletions in, *virG, sen, set*, and *gua* BA, is highly attenuated in humans. *Infect. Immun.* 68, 1034-1039.

Kristjansson, M., B. Viner, and J.N. Maslow. 1994. Polymicrobial and recurrent bacteremia with in a patient with AIDS. *Scand. J. Infect. Dis.* 26, 411-416.

Kruse, W. 1900. Ueberdie Ruhr als Volkskrankheit und ihrer erreger. *Dtsch. Med. Wochenschr.* 26, 637-639.

Levine, M.M., H.L. DuPont, S.B. Formal, R.B. Hornick, A. Takeuchi, E.J. Gangarosa, M.J. Snyder, and J.P. Libonati. 1973. Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. *J. Infect. Dis.* 127, 261-270.

Levine, M.M. 1986. Antimicrobial therapy for infectious diarrhoea. *Rev. Infect. Dis.* 8, S207-S216.

Levine, M.M. 1991. Shigellosis. In: Strickland GT, HuntersTropical Medicine. 7th Edition. Philadelphia, WB Saunders Co., 340-344.

Levine, O.S. and M.M. Levine. 1991. House flies (Musca domestica) as mechanical vectors of shigellosis. *Rev. Infect. Dis.* 13, 688-696.

Mata, L.J., E.J. Gangarosa, A. Caceres, D.R. Perera, and M.L. Mejicanos. 1970. Epidemic Shiga bacillus dysentery in Central America. etiologic investigations in Guatemala, 1969. *J. Infect. Dis.* 122, 170-180.

Mathan, V.I. and M.M. Mathan. 1991. Intestinal manifestations of invasive diarrhoeas and their diagnosis. *Rev. Infect. Dis.* 13 (Suppl 4), S311-313.

McCormack, B.A., A.M. Siber, and A.T. Maurerelli. 1998. Requirement of the *Shigella flexneri* virulent plasmid in the ability to induce trafficking of neutrophils across polarized monolayers of the intestinal epithelium. *Infect. Immun.* 66, 4237-4243.

McKee, K.T. Jr, T.M. Shields, P.R. Jenkins, J.M. Zenilman, and G. E. Glass. 2000. Application of a geographic information system to the tracing and control of an outbreak of shigellosis. *Clin. Inf. Dis.* 31, 728-733.

Mounier, J., V. Laurent, A. Hall, P. Fort, M.F. Carlier, P.J. Sansonetti, and C. Egile. 1999. Rho family GTPases control entry of *Shigella flexneri* into epithelial cells but not intracellular motility. *J. Cell Sci.* 112, 2069-2080.

Munshi, M.H., D.A. Sack, K. Halder, Z.U. Ahmed, M.M. Rahaman, and M.O. Murshed. 1987. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. *Lancet.* 2,419-421.

Murphy, G., L. Bodhidatta, P. Echeverria, S. Tansuphaswadikul, C.W. Hoge, S. Imlarp, and K. Tamura. 1993. Ciprofloxacin and loperamide in the treatment of bacillary dysentery. *Ann. Intern. Med.* 118, 582.

Nataro, J.P., J. Seriwatana, A. Fasano, D.R. Maneval, L.D. Guers, F. Noriega, F. Dubovsky, M.M. Levine, and J.G. Morris, Jr. 1995. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. *Infect. Immun.* 63, 4721-4728.

National Committee for Clinical Laboratory Standards. 1997. Performance standard antimicrobial disk susceptibility tests: approved standards. M2-A6, 6th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Nelson, J.D., H. Kusmiesz, and L.H. Jackson. 1976. Comparison of trimethoprim/ sulphamethoxazole and ampicillin therapy for shigellosis in ambulatory patients. *J. Pediatr.* 89, 491.

Noriega, F.R., F.M. Liao, S.B. Formal, A. Fasano, and M.M. Levine. 1995. Prevalence of *Shigella* enterotoxin 1 among *Shigella* clinical isolates of diverse serotypes. *J. Infect. Dis.* 172, 1408-1410.

Noriega, F.R., Losonsky, G., Lauderbaugh, C., Liao, F.M. Wang, J.Y., and M.M. Levine. 1996. Engineered *gua*B-A *vir*G Shigella flexneri 2a strain CVD 1205: construction, safety,immunogenecity, and potential efficacy as a mucosal vaccine. *Infect. Immun.* 64, 3055-3061.

Niyogi, S.K., U. Mitra, and P. Dutta. 2001. Changing pattern of serotypes and antimicrobial susceptibilities of *Shigella* species isolated from children in Calcutta, India. *Jpn. J. Infect. Dis.* 54, 121-122.

Niyogi, S.K., M. Vargas, and J. Vila. 2004. Prevalence of the *sat,set* and *sen* genes among diverse serotypes of *Shigella flexneri* strains isolated from patients with acute diarrhoea. *Clin. Microbiol. Infect.* 10, 574-576.

Nhieu, G.T. and P.J. Sansonetti. 1999. Mechanism of *Shigella* entry into epithelial cells. *Curr. Opin. Microbiol.* 2, 51-56.

Obituary. The New York Times 1957 26 Jan. sec. 19, 6.

Olsson-Liljequist, B. 1992. E-test as a routine MIC tool for reference work. *Diagn. Microbiol. Infect. Dis.* 15, 479-482.

Pal, S.C. 1984. Epidemic bacillary dysentery in West Bengal. *Lancet.* 1, 1462.

Panhotra, B.R., B. Desai, and P.L. Sharma. 1985. Nalidixic acid resistant *Shigella dysenteriae* type 1. *Lancet.* 1, 763.

Pozsgay, V., C. Chiayung, L. Pannel, J. Wolfe, J.B. Robbins, and R. Schneerson. 1999. Protein conjugates of synthetic saccharides elicit higher levels of serum IgG lipopolysaccharide antibodies in mice than do those of the O-specific polysaccharide from *Shigella dysenteriae type* 1. *Proc. Natl. Acad. Sci. USA* 96, 5194-5197.

Rahaman, M.M., M.M. Khan, K.M.S. Aziz, M.S. Islam, and A.K. Kibriya. 1975. An outbreak of dysentery caused by *Shigella dysenteriae* type 1 on a coral island in the bay of Bengal. *J. Infect. Dis.* 13215.

Robbins, J.B, C.Y. Chu, and R. Schneerson. 1992. Hypothesis for vaccine development : Protective immunity to enteric diseases caused by non-typhoidal *Salmonella* and *Shigellae* may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. *Clin. Infect. Dis.* 15, 346-361.

Robbins, J.B, R. Schneerson, and S.C. Szu. 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious disease by inactivating the inoculum. *J. Infect. Dis.* 171, 1378-1398.

Ross, S., G. Controno, and W. Khan. 1972. Resistance of shigellae to ampicillin and other antibiotic. *J. Am. Med. Assoc.* 221, 45.

Sack, R.B., M. Rahman, M. Yunus, and E.H. Khan. 1997 Antimicrobial resistance in organisms causing diarrhoeal disease. *Clin. Infect. Dis.* 24(suppl. 1), S102-105.

Salam, M.A. and M.L. Bennish. 1988. Therapy for shigellosis I randomized double-blind trial of nalidixic acid in childhood shigellosis. *J. Pediatr.* 113, 901-907.

Salam, M.A. and M.L. Bennish. 1991. Antimicrobial therapy for shigellosis. *Rev. Infect. Dis.* 13(Suppl 4), S332-S341.

Salam, M.A., C. Seas, W.A. Khan, and M.L. Bennish. 1995. Treatment of shigellosis: IV cefixime is ineffective in shigellosis in adults. *Ann. Intern. Med.* 123(7), 505-508.

Sansonetti, P.J. and J. Arondel. 1989. Construction and evaluation of a double mutant of *Shigella* flexneri as a candidate for oral vaccination against shigellosis. *Vaccine* 7, 443-450.

Sansonetti, P.J. and C. Egile. 1998. Molecular bases of epithelial cell invasion by *Shigella flexneri*? *Antonie Van Leeewenhoek.* 74, 191-197.

Sarkar, K., S. Ghosh, S.K. Niyogi, and S.K. Bhattacharya. 2003. *Shigella dysenteriae* type 1 with reduced susceptibility to fluoroquinolone. *Lancet.* 361, 785.

Schuch, R. and A.T. Maurelli. 1999. The mxi-Spa type III secretory pathway of *Shigella flexneri* requires an outer membrane lipoprotein MxiM, for invasion translocation. *Infect. Immun.* 67, 1982-1991.

Sen, D., P. Dutta, B.C. Deb, and S.C. Pal. 1988. Nalidixic acid resistant *Shigella dysenteriae* type 1 in Eastern India. *Lancet.* 2, 911.

Shears, P. 1996. *Shigella* infection. *Ann. Trop. Med. Parasitol.* 90, 105-114.

Shiga, K. 1898. Ueber den Erreger der Dysenterie in Japan. *Vorlaufige Mitteilung Zentralbal Bakteriol Microbiol Hyg.* 23, 599-600.

Shiga, K. 1898. Ueber den Dysenterie bacillus (*Bacillus dysenteriae*), *Zentralbl Bakteriol Parasitenkd Abt I Org.* 24, 817-824.

Shiga, K. 1906. Obsevation on the epidemiology of dysentery in Japan. *Philippine J. of Sci.* 1, 485-500.

Shiga, K. and K. Kanwa. 1950. Tokyo: Nihon Iji Shimpo Shuppanbu.

Shiga, K. 1936. The trend of prevention, therapy, and epidemiology of dysentery since the discovery of its causative organisms. *N. Engl. J. Med.* 215, 1205-1211.

Sieper, J., J. Braun, P. Wu, R. Hauer, and S. Laitko. 1993. The possible role of *Shigella* in sporadic enteric reactive arthritis. *Br. J. Rheumatol.* 32, 582-585.

Struelens, M.J., D. Patte, I. Kabir, A. Salam, S.K. Nath, and T. Butler. 1985. *Shigella* septicemia: prevalence, presentation, risk factors, and outcome. *J. Infect. Dis.* 152, 784-790.

Sur, D., S.K. Niyogi, S. Sur, K.K. Datta, Y. Takeda, G.B. Nair, and S.K. Bhattacharya. 2003. Multi-drug resistant *Shigelaa dysenteriae* type 1: Forerunners of a new epidemic strain in eastern India? *Emerg. Inf. Dis.* 9(3), 404-405.

Taylor, D., P. Echeverria, T. Pal, O. Sethabutr, S. Saiborisuth, S. Sricharmorn, B. Rowe, and J. Cross. 1986. The role of *Shigella* spp., enteroinvasive *Escherichia coli* and other enteropathogens as cause of childhood dysentery in Thailand. *J. Infect. Dis.* 153, 1132-1138.

Todd, C. 1904. On a dysentery toxin and antitoxin. *J. Hyg.* 4, 480-494.

Trofa, A.F., H. Ueno-Olsen, R. Oiwa, and M. Yoshikawa. 1999. Dr. Kiyoshi Shiga: Discoverer of the dysentery bacillus. *Clin. Infect. Dis.* 29, 1303-1306.

Vargas, M., J. Gascon, M.T. Jimenez, De Anta., and J. Vila. 1999. Prevalence of *Shigella* enterotoxins 1 and 2 among *Shigella* strains isolated from patients with travelers diarrhoea. *J. Clin. Microbiol.* 37, 3608-3611.

Varsano, I., T. Elditz-marcus, M. Nussinovch, and I. Elian. 1991. Comparative efficacy of ceftriaxone and ampicillin for treatment of severe shigellosis in children. *J. Pediatr.* 118, 627-632.

Venkatesan, M., P.C. Fernandez, J.M. Buysee, S.B. Formal, and T.L. Hale. 1991. Virulence phenotype and genetic characteristics of the T32-1STRATI *Shigella flexneri* 2a vaccine strain. *Vaccine* 9, 358-363.

Way, S.S., A.C. Borczuk, R. Dominit, and M.B. Goldberg. 1998. An essential role for gamma interferon in inate resistance to *Shigella flexneri* infection. *Infect. Immun.* 66, 1342-1348.

Way, S.S. and M.B. Goldberg. 1998. Clearence of *Shigella flexneri* occurs through a nitric oxide independent mechanism. *Infect. Immun.* 66, 3012-3016.

World Health Organization. 1987. Manual for the investigation of acute enteric infections, programme for control of diarrhoeal diseases. Geneva CDD/83.3. Rev. 1.

World Health Organization. 1995. The treatment of diarrhoea. A manual for physicians and other senior health workers. Geneva, Switzerland (WHO/CDR/95.3).

World Health Organization. 1997. New strategies for accelerating Shigella vaccine development. Weekly Epidemiol Record. 72, 73-80.

World Health Organization. 2001. Antimicrobial resistance in shigellosis, cholera and campylobacteriosis.WHO/CDS/CSR/DRS/2001.8. World Health Organization, Geneva, Switzerland.

Yavzori, M., D. Cohen, and N. Orr. 2002. Prevalence of the genes for *shigella* enterotoxin 1 and 2 among clinical isolates of *shigella* in Israel. *Epidemiol. Infect.* 128, 533-535.