24 Bacterial Enteropathogens

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KEY POINTS

- The principal bacterial agents of diarrhoeal diseases are *Vibrio cholerae*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. (especially *C. jejuni*) and a variety of enteropathogenic *Escherichia coli* strains, including the enterotoxigenic ETEC strains that are the main agents of travellers' diarrhoea.
- *H. pylori* causes acute and chronic non-autoimmune gastritis and is probably the commonest bacterial infection of mankind. Long-term infections cause up to 60% of gastric carcinomas and intestinal mucosa-associated lymphoid tumours (MALTomas).
- *Escherichia coli* is the major aerobic component of the normal intestinal flora. Most strains are commensals, but some pathotypes cause diarrhoeal disease. Over time, five different mechanisms have been described: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC); enterohaemorrhagic *E. coli* (EHEC); and enteroadhesive or enteroaggregative *E. coli* (EAEC or EAggEC).
- Campylobacters can produce both inflammatory diarrhoea and non-inflammatory diarrhoeas and are associated with Guillain-Barré syndrome. Data on *Campylobacter* infection in tropical areas is limited, partly because of the difficulty of isolating the organism with limited laboratory facilities.
- Cholera is characterized by severe watery diarrhoea leading to dehydration, electrolyte imbalance and hypovolaemia, with a mortality ranging from less than 1 to 40%. In the past decade, devastating epidemics of cholera have occurred in Angola, Ethiopia, Zimbabwe, Pakistan, Somalia, Sudan, Vietnam and Haiti.
- Dysentery has been a disease of poor and crowded communities throughout history and continues to be a major cause of morbidity and mortality in the tropics. *Sh. dysenteriae* and *Sh. flexneri* are responsible for most infections in the tropics. Shigellosis occurs both endemically and as epidemics.
- The wide diversity of organisms that may cause infections of the gastrointestinal tract and the differences in symptoms complicate accurate surveillance and diagnosis, especially in developing countries with limited access to modern laboratory procedures.

Introduction

Diarrhoeal diseases represent a major health problem in developing countries and are also a high risk to travellers who visit these countries. There is a high death toll from diarrhoeal disease estimated at about 2 million deaths per year.[1] Most of these deaths occur in children under 5 years of age. A rate of 3.2 episodes of diarrhoea/year per child has been reported, but can be higher in some settings. In addition to acute illness, repeated infections can lead to acute or chronic malnutrition and consequent effects on physical and mental development of children that may eventually translate into impairment of human fitness and productivity in adults.[2] Moreover, outbreaks of cholera, shigellosis and typhoid fever most often occur in resource-poor countries, adding to the burden of disease among the most vulnerable subpopulations.

The wide diversity of organisms that may cause infections of the gastrointestinal tract and the differences in symptoms complicate accurate surveillance and diagnosis, especially in developing countries with limited access to modern laboratory procedures. Among the principal bacterial agents of diarrhoeal diseases are *Vibrio cholerae* (cholera), a variety of *Salmonella* spp. and of *Shigella* spp., the agents of shigellosis (bacterial dysentery), *Campylobacter* spp. (especially *C. jejuni*) and a variety of enteropathogenic *Escherichia coli* strains, including the enterotoxigenic ETEC strains that are the main agents of travellers' diarrhoea. Bacterial diarrhoeas can also be caused by a variety of bacterial pathogens such as *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium difficile* or *Klebsiella*. In addition, infections may not result in gastroenteritis, for example, *Salmonella typhi* causes typhoid fever and *Helicobacter pylori* is associated with a range of symptoms from gastritis to malignancies.

In the gastrointestinal tract, bacteria that make up the normal flora are present from shortly after birth. The number of bacteria increases distally, with bacteria found in the stomach and small intestine in low numbers (10^2–10^4 colony forming units [cfu]/mL) which are usually transient(s) and the lower ileum and colon containing large numbers of bacteria (~10^{12} cfu/mL). In disease, the detection of the causative agent can be difficult and has led to several approaches to laboratory diagnosis and the assessment of causality. In this chapter, bacteria causing disease in the gastrointestinal tract are reviewed, pathogens that enter through the gut but have their major disease manifestations outside the gut, as in *Salmonella typhi* infections, are covered elsewhere.

Helicobacter pylori

Since the beginning of the 1900s, histopathologists have described spiral bacteria in the stomach. In 1983, Warren and Marshall were able to grow the bacterium originally named *Campylobacter pyloridis* and finally designated *Helicobacter pylori*.[3] It is now accepted that *H. pylori* causes acute and chronic non-autoimmune gastritis and is probably the commonest bacterial infection of mankind. It is responsible for up to 80% of

gastric and 95% of duodenal ulcers. In 1994, the International Agency for Research on Cancer classified *H. pylori* as a grade 1 carcinogen, the only bacterial agent of cancer. Long-term infections of 30–40 years cause up to 60% of gastric carcinomas. It is also associated with intestinal mucosa-associated lymphoid tumours (MALTomas).

Epidemiology

Infection with *H. pylori* is present all over the world.[3] In developed countries, approximately 10% of healthy individuals under 30 years of age have serological evidence of infection and this rises to 60% in those over 60. In developing countries, infection is highly prevalent and is acquired at a younger age. For example, in the Gambia, 46% of those under 5 years and in Peru, 48% of children under 12 years had evidence of infection.[4,5] In most developing countries, virtually 100% are seropositive by early childhood.[6] Infection is usually acquired in the first 5 years of life but improving hygienic and socioeconomic conditions in some developing countries have led to a decreased rate of acquisition. Humans are the major reservoir for *H. pylori*. The bacterium has been grown or its genome detected in saliva, dental plaque, vomitus, gastric juice and faeces.[7] The relative importance of different modes of transmission are unclear. Person-to-person spread via endoscopes, pH electrodes or nasogastric feeding tubes has been documented. Close contact clearly promotes spread, e.g. families of infected children have a higher incidence of infection, as have those who are occupationally exposed.[8,9] Family clusters of infection are related to socioeconomic status[10] and infection is readily transmitted between siblings.[11] The faecal–oral route is the most likely mode of spread and *H. pylori* DNA and antigen have been detected in faeces. Inter-oral spread has also been proposed, with the oral cavity considered a permanent reservoir of *H. pylori*.[12] The domestic fly (*Musca domestica*) can become colonized by *H. pylori* and *H. pylori* DNA has been detected in houseflies from three continents.[13] This raises the possibility of fly contamination of food leading to food-borne infection. *H. pylori* does not grow in foods but does survive in a cool, moist and non-acidic environment. Water-borne spread has also been suggested as a major route in developing countries.[5] Finally, some animal species, including the macaque, sheep and pig, have been shown to harbour *H. pylori*, suggesting the possibility of zoonotic spread.[7] A number of other *Helicobacter* species have been detected in a variety of animals, but only *H. heilmannii* is found in the human stomach.

Microbiology

H. pylori is a sinusoidal Gram-negative bacterium, 3.5 μm long by 0.5–1 μm in diameter (Figure 24.1), with a smooth surface and four to six sheathed flagella with terminal bulbs. The bacterium produces a urease and is well adapted to living beneath the mucous layer attached to the surface of gastric enterocytes. *H. pylori* is fastidious and slow-growing. It requires enriched selective media for isolation from clinical specimens. Growth is optimal at 37°C under humidified microaerophilic conditions in 10% carbon dioxide and takes 4–6 days.

Pathogenesis

A causal association has been demonstrated for antral non-autoimmune (type B) gastritis both in adults and in children.[3,14,15] There is also a strong association between *H. pylori* and peptic ulceration. Infection can be established with

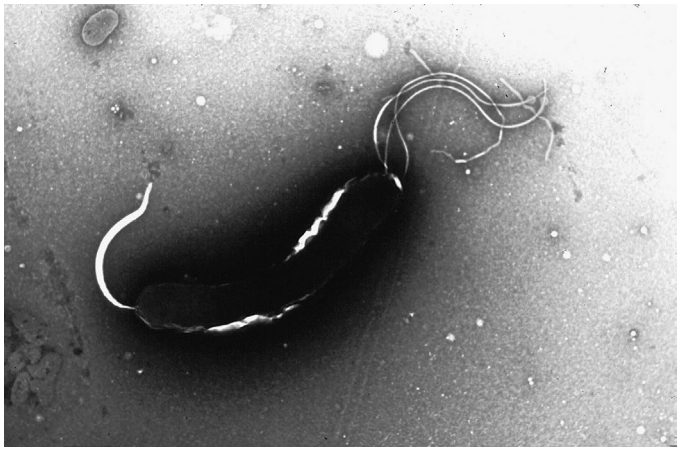

Figure 24.1 Negative-stain electron micrograph of *Helicobacter pylori* showing sheathed flagella with a terminal bulb.

doses of between 10^5–10^9 cfu. *H. pylori* is able to survive an acidic gastric pH to penetrate the mucus covering the gastric epithelial cells, where it can exist free or attach to the epithelial cells (Figure 24.2). The bacterium's spiral morphology and flagella are important for pathogenesis.[9] *H. pylori* makes an enzyme, urease, which breaks down urea to ammonia which helps to neutralize the acidic pH, a cytotoxin which causes vacuolation (Vac A), and a protease which hydrolyses mucus and other factors which stimulate gastric acid secretion. Recently it has been found that strains of *H. pylori* that have a 40 kb pathogenicity island called the *cag* pathogenicity island (PAI) are more likely to produce inflammation. The *cag* PAI region encodes a secretion system (type IV) that transports a protein, cag, across both bacterial membranes and injects it into host cells. Cag, also encoded in the PAI, induces the secretion of pro-inflammatory cytokines.[16] CagA interacts with the tumour suppressor apoptosis-stimulating protein of p53–2 (ASPP2), promoting p53 degradation. The pathogenesis of MALToma production appears to involve chronic antigenic stimulation and elimination of *H. pylori* is associated with cure of the lymphomas.

Infected individuals mount a systemic and local humoral immune response to the bacterium. The immune response to

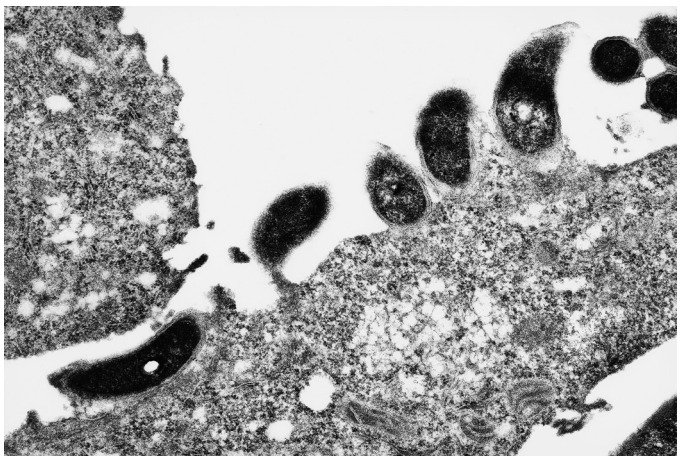

Figure 24.2 Thin-section electron micrograph of *H. pylori* intimately attached to a gastric enterocyte.

Helicobacter pylori is multifaceted, involving responses that are both protective and damaging to the host. The innate and the adaptive immune responses lead to damaging inflammatory responses, but these responses may fail, allowing for persistence of infection.[17]

Pathology

Examination of biopsies in antral gastritis reveals *H. pylori* in close apposition to the gastric mucosa, which shows an infiltrate with mono- and polymorphonuclear leukocytes. *H. pylori* and evidence of inflammation may also be found in areas of gastric metaplasia in the oesophagus (Barrett's oesophagus) or duodenum. Duodenal ulceration is associated with chronic antral gastritis. *H. pylori* can be detected in both antrum and duodenal ulcer tissue, but it will not colonize the duodenum except in areas of duodenal metaplasia.[18]

Clinical Features

Although a large proportion of infections are asymptomatic, chronic epigastric pain is common in infected individuals. In sub-Saharan Africa, non-ulcerous dyspepsia and duodenal ulcer are the most common causes of epigastric pain.[19] Infection with *H. pylori* was found in 88% of adult Malawians undergoing gastroscopy for chronic epigastric pain. Other features associated with *H. pylori* gastritis include nausea, vomiting and flatulence. Similar features may also be seen in children with *H. pylori* infection.[15]

Diagnosis

Specific diagnosis may be reached by invasive or non-invasive techniques (Table 24.1).

Invasive Techniques. Endoscopic biopsies from the antrum, duodenal ulcer(s) or other areas of potential colonization are examined by culture and histology and for urease activity. Two biopsy specimens from the antrum are sufficient to detect *H. pylori*.[19] Histological samples may be stained by Giemsa, silver impregnation or acridine orange for detection of *H. pylori*.

For culture, biopsy specimens are either rolled on the surface of an appropriate culture medium (e.g. brain–heart infusion-enriched Columbia blood agar incorporating Skirrow's antibiotics) or homogenized and similarly applied. In tropical countries it is advisable to incorporate an antifungal such as amphotericin B into the medium. A '1 minute' urease test, in which the biopsy is immersed in a urea (10% deionized water) solution containing a pH indicator (phenol red), has proved highly sensitive and specific.[19]

Non-invasive Techniques. Detection of antibody to *H. pylori* in serum or saliva is possible using an enzyme-linked immunosorbent assay (ELISA). Such tests have proved highly sensitive[14,15] but the specificity is variable, since it is possible to detect antibody in those who are no longer infected.[19]

Breath tests which involve administering [^{13}C] urea and measuring the release of the isotope in the patient's breath have been useful but require the availability of the isotope and are expensive. They depend upon the presence of *H. pylori* urease, which hydrolyses the urea with release of $^{13}CO_2$. Antigen-capture ELISAs have been developed for detection of *H. pylori* in stool. It has proved sensitive and specific[20] and is useful as a test of cure. It is rapid and easy to carry out and in the case of monoclonal antibody-based tests, is at least equivalent to the urea breath test[21] but is infrequently used in developing countries because of the cost.

Management and Treatment

In developing countries, non-ulcer dyspepsia may not be treated other than by symptomatic management. *H. pylori* is susceptible in vitro to a wide range of antimicrobials, including ampicillin, quinolones, cephalosporins, nitroimidazoles and macrolides, but all fail as monotherapy in vivo. Current recommendations for first-line empirical therapy in areas with low clarithromycin resistance are clarithromycin containing regimens with proton pump inhibitors with and without amoxicillin or metronidazole and where resistance is high, bismuth-containing quadruple therapy.[21] Unfortunately, resistance of *H. pylori* to metronidazole is increasing. In cases of failure, levofloxacin is used, but levofloxacin resistance is also reported.[21]

Complications

In Gambian children, an association between *H. pylori* and chronic diarrhoea and malnutrition has been described.[4] *H. pylori* gastritis was associated with protein-losing enteropathy in South African children.[22] Co-infection with *H. pylori* and *Vibrio cholerae* 01 was found frequently in Peruvian children and elderly adults, suggesting that hypochlorhydria induced by acute and chronic *H. pylori* infection might increase susceptibility to cholera.[23] Finally, epidemiological studies have suggested a link between current infection with *H. pylori* and atherothrombogenesis.[24]

TABLE 24.1 Invasive and Non-Invasive Tests for the Diagnosis of *H. pylori* Infection

Test	Sensitivity (%)	Specificity (%)	Cost	Comment
Non-Invasive				
Antibody detection – ELISA	84–95	80–95	+	Can be used for proof of cure
Antibody detection – rapid	60–75	88–92	+	Can be used for proof of cure
Stool antigen detection	90–100	92–95	++	Becomes rapidly negative after therapy
^{13}C breath test	90–96	99	+++	Needs specialized equipment
^{14}C breath test	90–96	99	+++	Needs specialized equipment
Invasive				
Histology	80–90	93–100	+++	Takes time
Culture	75–90	100	+	Takes 3–4 days
Urease	85–95	99	+	Rapid test
Gram or other stain	75–90	80–90	+	Rapid but not ideal
PCR	95–100	95–99	+++	Very sensitive, takes 4–5 hours

TABLE 24.2 *Escherichia coli* and Gastroenteritis

Pathogenicity Type	Site of Action	Associated Serogroups	Pathogenicity Genes/ Products	Acute or Chronic Diarrhoea	Antibiotic Therapy Needed
ETEC	Small bowel (secretory)	O6, O8,O15, O2O, O25, O128, O139, O148, O153, O159	CFA, LT, ST	Acute	No
EIEC	Large bowel (inflammatory)	O28, O29, O124, O136, O143	*ipa*, *ial*, EIEC	Acute	Not usually
EPEC	Small bowel (osmotic)	O55, O86, O111, O119, O125, O126, O127, O128, O142	LEE (EspA, intimin, Tir)	Chronic	Yes
EHEC	Large bowel (inflammatory)	O26, O111, O118, O138, O157	LEE, EHEC, VT1, VT2	Acute	No
EAggEC	Large bowel (inflammatory)	O44, O111, O126 *but* most are non-groupable	EAggEC adhesin, EAST-1	Acute and chronic	Yes

CFA, colonization factor antigen; EAST, EAggEC heat-stable toxin; *ipa* and *ial*, invasion-associated loci; LEE, locus of enterocyte effacement; LT, heat-labile toxin; ST, heat-stable toxin; Tir, translocatable intimin receptor; VT, vero (or Shiga-like) toxin.

Prevention and Control

Infection with *H. pylori* is ubiquitous throughout the world. Until more is known about the mode of spread, pathogenesis and immunity, prevention and control are impossible. The decrease in infection in children in developed countries suggests that improving socioeconomic and hygienic conditions should decrease the burden of infection. While no vaccines are currently licensed; new treatments/regimens have been shown to increase the eradication rates.[25]

Escherichia coli

Escherichia coli is the major aerobic component of the normal intestinal flora (~10^7 cfu/mL). Most strains are commensals, but some pathotypes cause diarrhoeal disease. The strains of *E. coli* causing diarrhoea were originally called enteropathogenic *E. coli* (EPEC), but over time five different mechanisms have been described: EPEC, enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) and enteroadhesive or enteroaggregative *E. coli* (EAEC or EAggEC).

E. coli was first described as a cause of gastroenteritis by its association with outbreaks of diarrhoea in infants.[26,27] This was done by showing that all infants were excreting strains of *E. coli* with the same O or somatic antigen. Different O antigens were associated with the different enteropathogenetic mechanisms of *E. coli* (Table 24.2) and while O serogrouping was used to classify EPEC until the 1980s, more recently molecular methods have been used to classify the diarrhoeagenic *E. coli*.

ENTEROTOXIGENIC *E. COLI* (ETEC)

Epidemiology

ETEC have been described worldwide, but with higher rates in some developing countries. In addition, they are a major cause of traveller's diarrhoea. In community-based studies in Bangladesh, ETEC were responsible for 15–20% of cases of diarrhoea.[28] In hospital-based studies, ETEC are a common bacterial cause of gastroenteritis. ETEC infections occur throughout the year but are most common in the wet season. Spread is by the faecal–oral route, either directly or indirectly via food or water. Infants are at particular risk at weaning. The infective dose is high (10^6–10^{10} cfu). In a recent review focusing on ETEC epidemiology with regard to toxin and colonization factor antigen (CFA) production, 60% of isolates expressed heat-labile toxin (LT) either alone (27%) or in combination with the heat-stable toxin (ST, 33%). CFA/I-expressing strains were common in all regions (17%), as were ETEC expressing CFA/II (9%) and IV (18%). There is a marked variation in toxins and CFs across regions and populations.[29]

Pathogenesis

ETEC colonize the small intestine and elaborate one heat-labile toxin (LT) or heat-stable toxin (ST) or both. ETEC colonize the small intestine by means of adhesive fimbriae or pili called colonization factor antigens or CFAs (Figure 24.3), which bind to specific receptors on the enterocyte surface. The bacteria then release their toxins. LT is a subunit toxin with a structure and mode of action similar to cholera toxin. Subunit B binds to GM_1 ganglioside on the enterocyte surface and allows subunit A to activate adenylate cyclase inside the enterocyte. The raised intracellular cyclic AMP concentration causes active C1− secretion from villous crypt cells. The net effect is that a large fluid load enters the colon and a voluminous watery stool is produced. ST is a low-molecular-weight protein that activates guanylate cyclase. This results in secretion of fluid and electrolytes into the intestinal lumen. There are no specific histopathological changes to be seen in the small-intestinal mucosa and no

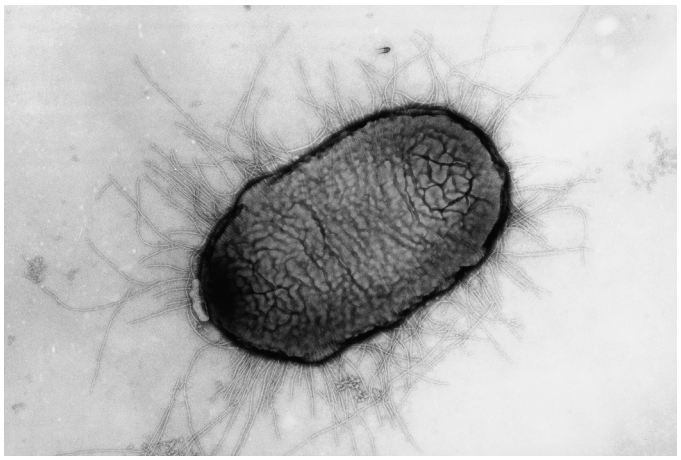

Figure 24.3 Negative-stain electron micrograph of an enterotoxigenic *E. coli* covered with fimbriae.

evidence of inflammation. The genes encoding fimbriae, LT and antibiotic resistance are carried on plasmids in ETEC.

Clinical Features

The incubation period is 1–2 days, with anorexia, vomiting and abdominal cramps in 25% of patients. The diarrhoea is explosive, voluminous and watery, up to 10 times a day. The illness is self-limiting and usually lasts 1–5 days in well-nourished persons, but up to 3 weeks in malnourished children. Dehydration is the major complication, which, in a study in Bangladesh, was seen in 46% of adults and 16% of children.[28]

Diagnosis

Specific diagnosis depends upon culture of *E. coli* from faeces and detection of pathogenicity genes (CFA, LT, ST) by PCR, or their gene products by ELISA, immunoprecipitation or bioassay. Even though molecular methods are becoming more available,[30] ETEC diagnosis in developing countries is rarely done outside of research studies.

Treatment

The mainstay of treatment is the assessment of dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown to shorten the course of illness and duration of excretion of ETEC in adults in endemic areas and in traveller's diarrhoea. The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are currently fluoroquinolones or azithromycin, with an emerging role for rifaximin. Oral rifaximin, a semisynthetic rifamycin derivative, is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveller's diarrhoea. Rifaximin was significantly more effective than placebo and no less effective than ciprofloxacin in reducing the duration of diarrhoea. While rifaximin is effective in patients with *E. coli*-predominant traveller's diarrhoea, it appears ineffective in patients infected with inflammatory or invasive enteropathogens.[31]

Prevention

Antibodies against the LT and major CFs of ETEC provide protection against LT-producing ETEC expressing homologous CFs. Oral inactivated vaccines consisting of toxin antigen and whole cells, i.e. the licensed recombinant cholera B subunit (rCTB)-WC cholera vaccine Dukoral and candidate ETEC vaccines have been developed. In different trials, the rCTB-WC cholera vaccine provided high (85–100%) short-term protection. An oral ETEC vaccine consisting of rCTB and formalin-inactivated *E. coli* bacteria expressing major CFs has been shown to be safe, immunogenic and effective against severe diarrhoea in American travellers but not against ETEC diarrhoea in young children in Egypt. A modified ETEC vaccine consisting of recombinant *E. coli* strains overexpressing the major CFs and a more LT-like hybrid toxoid called LCTBA, have been developed and are being tested.[32]

ENTEROINVASIVE *E. COLI* (EIEC)

EIEC produce inflammatory diarrhoea by invading and killing colonic enterocytes (Figure 24.4). They resemble shigellae in O antigens, in being non-motile and have similar pathogenicity genes on a large plasmid that encode surface proteins mediating invasion into cells. Infection is less common than shigellosis.

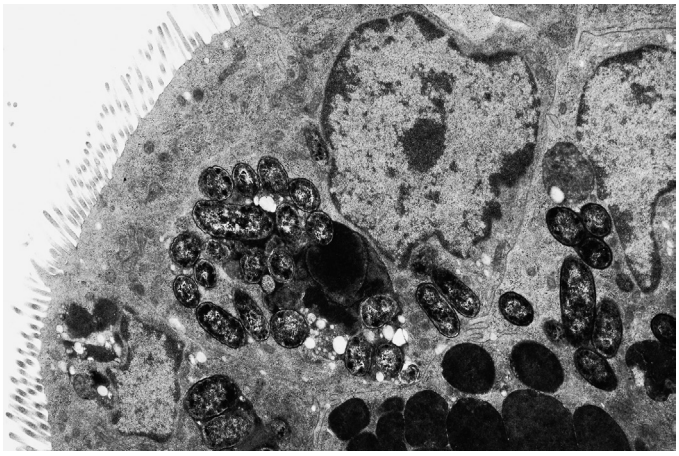

Figure 24.4 Thin-section electron micrograph of colonic enterocytes showing enteroinvasive *E. coli* that have invaded into the cells.

For example, EIEC were responsible for 4.2% of episodes of endemic diarrhoea in children in Thailand and shigellae for 23%.[32] Infection is uncommon in children under 1 year old but can be a cause of traveller's diarrhoea. The clinical features of EIEC infection are similar to those of shigellae. Diagnosis is by stool culture and detection of EIEC pathogenicity genes by DNA hybridization or PCR.[30] Antimicrobial chemotherapy is usually not indicated and no vaccine is currently available for prevention.

ENTEROPATHOGENIC *E. COLI* (EPEC)

In the early 1970s, when the pathogenesis of ETEC and EIEC had been defined, it became apparent that a large number of the classical O serogroups did not elaborate LT or ST, nor were they invasive. However, they were able to produce diarrhoea in volunteers.[33] Since these were the original classical O serogroups that caused outbreaks of infantile diarrhoea, they were termed enteropathogenic *E. coli*. EPEC were originally defined by serogroups. However, it became clear that serogroup/serotype designation over-diagnosed EPEC. Subsequently, they were defined by their characteristic localized adherence pattern in tissue-cultured cells. Currently, they are identified mainly based on the presence of specific virulence genes.[34]

Epidemiology

The first infections with EPEC were described in the UK and the USA in the 1940s and 1950s in epidemics of infantile diarrhoea. They are now considered a cause of sporadic disease in young children. EPEC has also been associated with traveller's diarrhoea. Transmission is by the faecal–oral route, either directly or in food or water. The infective dose appears to be low ($<10^4$ cfu).

Pathogenesis

EPEC have an unusual pathogenetic mechanism, in that they synthesize, secrete and insert their own receptor into host cell membranes. The ingested EPEC adhere to the mucus overlying the small-intestinal enterocytes using fimbriae. On contact with enterocytes, pathogenicity island (called the locus of enterocyte effacement: LEE)-associated genes are activated. This induces the formation of a type III secretion system that delivers effector molecules across both bacterial membranes and through a

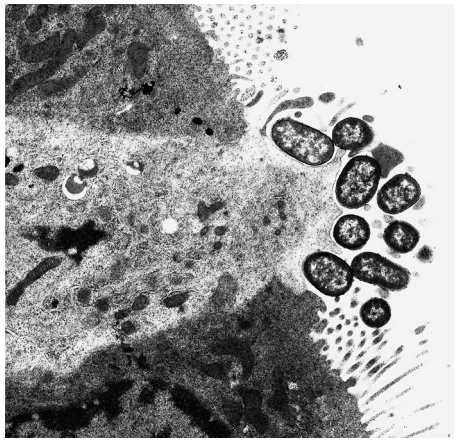

Figure 24.5 Thin-section electron micrograph of duodenal mucosa showing loss of brush border and intimately attached enteropathogenic *E. coli* (attaching/effacement).

pilus-like structure into the enterocyte.[35] Secreted effectors include Tir (translocatable intimin receptor), which becomes inserted in the enterocyte membrane. This has affinity for intimin, a surface protein on the EPEC, which then mediates the intimate attachment of EPEC to the enterocyte surface.[36] The brush border is lost with a flattening of the surface and re-arrangement of enterocyte cytoskeleton to form a cup or pedestal where the bacterium is attached, an attaching effacing lesion (Figure 24.5). Although the process is maximal in the small intestine, it can occur throughout the gastrointestinal tract. The net result is that large areas for absorption of nutrients are lost. In addition, because the disaccharidase enzymes are integral proteins in the microvillous membrane, levels of these enzymes are markedly depressed.[37] The disaccharides sucrose, lactose and maltose in the diet must be hydrolysed for monosaccharides to be absorbed. Because of loss of the brush border, the disaccharides cannot be cleaved and are thus not absorbed. They pass to the colon and cause a non-inflammatory osmotic diarrhoea, although in some cases there also appears to be a secretory component.

Clinical Features and Diagnosis

EPEC tend to produce severe and prolonged diarrhoea, which may remit and relapse. There is initially vomiting, with fever and profuse diarrhoea with mucus but no blood. Fatality rates in early epidemics ranged from 30% to 50%, but with oral rehydration and antibiotic therapy mortality rates have decreased to less than 8%.[38]

Diagnosis was previously made by stool culture on semi-selective media such as MacConkey agar followed by serogrouping on non-mucoid lactose fermenting colonies identified to be *E. coli* with specific antisera. Recently, serogrouping has been replaced by a combination of culture and molecular methods to identify specific genes. Recent epidemiological studies indicate that atypical EPEC (aEPEC) is more prevalent than typical EPEC (tEPEC) in both developed and developing countries and that aEPEC is important in both paediatric endemic diarrhoea and diarrhoeal outbreaks.[34]

Treatment and Prevention

The initial treatment is rehydration. Because the diarrhoea can be prolonged, enteral or parenteral nutrition and antibiotics may be indicated.[39] Resistance to most antibiotics has been observed in EPEC and probiotics have been suggested to restore gut health. A vaccine is not currently available for prevention of infection.

ENTEROHAEMORRHAGIC *E. COLI* (EHEC)

EHEC were first described in 1983, when they were linked to cases of haemorrhagic colitis and haemolytic–uraemic syndrome.[40,41] Infections were caused by a newly recognized serogroup, *E. coli* O157. Subsequently, a number of other *E. coli* O serogroups and other coliforms (*Enterobacter cloacae* and *Citrobacter freundii*), have been recognized to cause similar disease in humans. The recognition of the production of specific cytotoxins by EHEC, led to additional terms, such as verocytotoxin producing *E. coli* (VTEC) or Shiga toxin producing *E. coli* (STEC).

Epidemiology

Infections with EHEC were initially described in industrialized countries, where they cause outbreaks of infection usually as the result of the consumption of incompletely cooked beef or pork, or following contact with animals such as in petting zoos.[42] EHEC can be part of the normal enteric flora of cattle, pigs, sheep, goats, cats and dogs, in which they cause no disease.

Symptomatic infections are not as frequently reported from developing countries,[43] except in specific settings, such as large outbreaks of haemorrhagic colitis caused by EHEC associated with animal contamination of open water supplies in drought-affected areas in Swaziland and in Cameroon.[44,45] However, because the infective dose is low ($<10^2$ cfu), person-to-person spread also occurs.

In 2011, large outbreaks of haemolytic-uraemic syndrome in Europe drew attention to an unusual strain of EAHEC O104:H4 as an emerging *E. coli* pathotype that is endemic in Central Africa and has spread to Europe and Asia. EAHEC strains have evolved from enteroaggregative *E. coli* by uptake of a Shiga toxin 2a (Stx2a)-encoding bacteriophage. Except for Stx2a, no other EHEC-specific virulence markers including the locus of enterocyte effacement are present in EAHEC strains. EAHEC O104:H4 colonizes humans through aggregative adherence fimbrial pili encoded by the enteroaggregative *E. coli* plasmid. The aggregative adherence fimbrial colonization mechanism substitutes for the locus of enterocyte effacement functions for bacterial adherence and delivery of Stx2a into the human intestine, resulting clinically in haemolytic-uraemic syndrome.[46]

Pathogenesis

EHEC produce attaching effacement, limited to the terminal ileum and colon and similar genes to those in the EPEC LEE pathogenicity island. In addition, they release one or both of the toxins originally named verocytotoxins (VT) 1 and 2. These toxins are now called Shiga-like toxins (SLT) 1 and 2; they inhibit protein synthesis and are cytotoxic.[47] They are subunit toxins that bind to globoside receptors (the P-blood group antigen) on cells. The receptors are more densely expressed on renal endothelial cells and in children. In the colon they kill enterocytes, leading to an inflammatory haemorrhagic colitis. If they enter the systemic circulation, they can damage renal endothelial cells and precipitate the haemolytic–uraemic syndrome.

Clinical Features

Haemorrhagic colitis presents with abdominal cramps and watery diarrhoea that is followed by a haemorrhagic discharge resembling a colonic bleed. There is rarely an accompanying fever. Haemolytic-uraemic syndrome (HUS) is one of the commonest, if not the most common, cause of acute renal failure in childhood in industrialized countries. It has also been reported from developing countries, but is less frequent even though EHEC are more common in HUS than shigellae.[48] HUS presents with acute renal failure, thrombocytopenia, coagulopathy and evidence of microangiopathic haemolytic anaemia. With peritoneal dialysis, the fatality rate falls from 50% to less than 10%.

Diagnosis

The first strains of *E. coli* associated with haemorrhagic colitis and HUS were of serogroup O157; and sorbitol non-fermenters. Thus, serogrouping and sorbitol MacConkey agar are used to diagnose infections. However, other serogroups (Table 24.1) are also implicated. The toxins SLT1 and SLT2 are transferable between bacteria on promiscuous bacteriophages. Thus, specific diagnosis depends upon detection of SLT or its genes (by DNA hybridization or PCR) or of EHEC plasmid encoded fimbrial adhesin genes.[30] Excretion of EHEC beyond the period of diarrhoea is short-lived. For retrospective diagnosis it is possible to detect serum antibody to SLT.[49]

Treatment and Prevention

The treatment of haemorrhagic colitis is essentially treatment of dehydration. Antibiotics have no role and in some cases (as with *Sh. dysenteriae* 1) may increase the risk of complications.[50] For haemolytic–uraemic syndrome, peritoneal dialysis is the most important intervention. No vaccine is currently available. Prevention requires meticulous attention to food safety and to high levels of hygiene where contact with animals, particularly ruminants, is expected.

ENTEROAGGREGATIVE *E. COLI* (EAGGEC OR EAEC)

EAggEC are the most recently discovered pathogenic group.[51] As well as sporadic cases, outbreaks of EAggEC-caused diarrhoea have been described. EAggEC is a cause of acute diarrhoeal illness among children residing in both developing and developed regions, adults and persons with HIV infection residing in developing regions and travellers to developing regions in both developing and industrialized regions. The definition of EAggEC is the ability of the microorganism to adhere to epithelial cells such as HEp-2 in a very characteristic 'stacked-brick' pattern. Although many studies searching for specific virulence factor(s) unique for this category of DEC have been published it is still unknown why the EAggEC cause persistent diarrhoea. The gold standard for identification of EAggEC includes isolation of the agent and an adherence assay using tissue culture, *viz.* HEp-2 cells.

EAggEC strains are relatively heterogeneous and limited numbers of studies that examined the independent roles of the many putative EAggEC virulence genes in acute diarrhoeal illness have led to no firm conclusions regarding class-wide pathogenetic mechanisms. Molecular targets used for diagnosis by PCR include aaf, aggR, aaiC and aatA.[52]

EAggEC can cause both acute and persistent diarrhoea. In a survey of EAggEC infection in India, the most notable clinical features were fever, vomiting, overt blood in the stool and a mean duration of diarrhoea of 17 days.[53] Although not all EAggEC infections result in symptomatic illness, the most commonly reported symptoms are watery diarrhoea with or without blood and mucus, abdominal pain, nausea, vomiting and low-grade fever. Electron microscopy of infected small- and large-intestinal mucosa, from children between 3 and 190 months, cultured with several different EAggEC strains, reveals bacteria in a thick mucus layer above the intact enterocyte brush border. In the colon, EAggEC elicits inflammatory mediators and produces cytotoxic effects such as microvillus vesiculation, enlarged crypt openings and increased epithelial cell extrusion. Numerous putative virulence factors, a yersiniabactin system, a complex carbohydrate-specific lectin, enterotoxins and cytotoxins have been identified.[54]

One of the major difficulties in identifying the mechanism of pathogenesis for EAggEC is the diversity and the heterogeneity of EAggEC strains. No virulence factor has been identified as common to all EAggEC strains. EAggEC pathogenesis is therefore a complex host–pathogen interaction that involves host genetic susceptibility, heterogeneity of virulence among EAggEC strains and the amount of bacteria ingested by the infected host.

Campylobacter jejuni

The genus *Campylobacter* is a major cause of gastroenteritis in both developed and developing countries. Although *C. fetus* was recognized as an opportunist pathogen as early as 1947, the full role of *Campylobacter* species as major enteric pathogens was not realized until appropriate selective media were devised.[55]

A related genus, *Arcobacter* (principally *A. butzleri*), is increasingly recognized as an enteropathogen with similar pathogenic potential to *Campylobacter*.[56]

Epidemiology

Although *C. jejuni* continues to be the leading cause of bacterial gastroenteritis in humans worldwide, advances in molecular biology and the development of innovative culture methodologies have led to the detection and isolation of a range of under-recognized and nutritionally fastidious *Campylobacter* spp., including *C. concisus*, *C. upsaliensis* and *C. ureolyticus*. These emerging *Campylobacter* spp. have been associated with a range of gastrointestinal diseases, particularly gastroenteritis, inflammatory bowel disease and periodontitis. Over 90% of cases of *Campylobacter* gastroenteritis are associated with *C. jejuni*.

All *Campylobacter* spp. can be normally present in the gastrointestinal tract of domestic and wild animals and birds, which act as the major reservoir for infection. *C. lari*, in particular, can be part of the normal intestinal flora of birds. Campylobacters can survive for 2–5 weeks in cow's milk or water kept at 4°C but they do not multiply. Infection is spread faeco-orally, human-to-human or animal-to-human, either directly or indirectly in food and water.

- *Animal-to-human*. Close contact with animals increases the risk of infection
- *Human-to human*. Transmission may occur from infected individuals or from convalescent carriers, especially young

children. Epidemics of infection can occur in nurseries or paediatric wards
- *Food*. Contamination can occur during preparation of food from the animal's intestinal content(s) or by incomplete cooking
- *Milk*. Consumption of raw unpasteurized milk is strongly associated with illness, as is contamination of bottled milk following attack by birds[57]
- *Water*. Excreta from wild and domesticated animals can contaminate surface water and water-borne transmission is important in developing countries.

The incubation period is 2–5 days with an infective dose of 500 cfu. The median duration of excretion of *C. jejuni* following cessation of diarrhoea is 2–3 weeks. Infection is most common in those under 1 year old, with a decrease in attack rate with increasing age. Data on *Campylobacter* infection in tropical areas are limited, partly because of the difficulty of isolating the organism with limited laboratory facilities. Its prevalence has been shown from studies in India, Egypt and southern Africa.[58–60] *Campylobacter* infection has been associated with Guillain–Barré syndrome, an autoimmune polyneuropathy affecting the peripheral nervous system.[61]

Bacteriology

Campylobacters are Gram-negative bacteria with a single polar flagellum (Figure 24.6). They are spiral or bent rods, 0.2–0.5 μm in diameter and 1.5–3.5 μm long. They are thermophilic and will grow at 42°C but prefer a microaerophilic atmosphere. *C. jejuni* can hydrolyse hippurate, which distinguishes it from *C. coli* and *C. lari*. *C. coli* is sensitive to nalidixic acid but *C. lari* is resistant. All can be cultivated on simple media, but isolation can be facilitated with the use of antibiotics, oxygen quenching agents or a low oxygen atmosphere.[61]

Pathogenesis

Campylobacters can produce both inflammatory diarrhoea and non-inflammatory diarrhoeas. How Campylobacters cause diarrhoea is unclear but it does involve attachment to the intestinal mucosa and is also dependent on motility by means of flagella.[61] Other factors include iron acquisition, invasion of enterocytes and possibly toxin production.

Figure 24.6 Negative-stain electron micrograph of *Campylobacter jejuni*.

Immunity to infection appears to be acquired following one or more infections, but duration of immunity is unknown. Following infection, serum and secretory antibodies to *Campylobacter* flagella, enterotoxin, lipopolysaccharide and other surface antigens that are involved in attachment are produced. In developing countries, antibodies are acquired in early life[62,63] – perhaps because of continuous exposure from animals. This higher exposure may account for greater asymptomatic infection and the lower prevalence of disease in adults in developing countries compared with developed countries. In a small proportion of those infected, usually the immunocompromised, bacteria translocate from the intestinal lumen, causing bacteraemia. In Guillain-Barré syndrome, the pathogenesis is due to a form of molecular mimicry, where *Campylobacter* contains ganglioside-like epitopes in the lipopolysaccharide moiety that elicit autoantibodies reacting with peripheral nerve targets.

Pathology

In the more severe dysentery-like illness, inflammatory infiltrates into the lamina propria and crypt abscesses can be seen in the rectal, colonic and terminal ileal mucosa.

Clinical Features

In developing countries, *Campylobacter* enteritis is generally less severe than that in developed countries. It is more likely to be of the non-inflammatory type, without fever or bloody diarrhoea.[63] However, severe bloody diarrhoea resembling bacillary dysentery can occur and also occurs in travellers acquiring infection in developing countries. In general, diarrhoea is self-limiting and resolves in 2–7 days.

Disseminated infection can occur and predisposing factors include: malnutrition, hepatic dysfunction, malignancy, diabetes mellitus, renal failure and immunosuppression. Extraintestinal and rare forms of infection include: asymptomatic bacteraemia, meningitis, deep abscesses and cholecystitis. Reactive arthritis may follow campylobacteriosis enteritis in genetically susceptible individuals (HLA-B27). *Campylobacter* enteritis is one of the commonest precipitating causes of Guillain–Barré syndrome.

Diagnosis

The features of *Campylobacter* infection are not sufficiently distinctive to make a clinical diagnosis. Examination of faecal smears by Gram stain or dark-field microscopy can provide a rapid presumptive diagnosis. Where laboratory facilities are not optimal, this may be the best diagnostic tool. However, the basis of specific diagnosis is isolation of the bacteria from faeces. *Campylobacter* spp. will grow on most basal media, especially if lysed blood is incorporated. Common media include Columbia Blood Agar base, Butzler's medium and Preston medium. In order to make the media selective, antibiotics such as trimethoprim are incorporated.[64] More recently, a blood-free medium containing charcoal, cefoperazone and amphotericin B has been developed. Culture is usually at 42°C (to inhibit gut commensals) and in a microaerophilic atmosphere. Culture plates and swabs should be kept out of the light prior to use since *Campylobacter* spp. are rapidly killed by free radicals generated by ultraviolet irradiation.

Treatment

Severe watery diarrhoea is treated by adequate rehydration. Cases of severe dysentery or disseminated infection will need antimicrobial chemotherapy. *C. jejuni* is usually sensitive to erythromycin, but increased resistance has been reported and quinolones such as ciprofloxacin may be required.

Prevention and Control

There is no vaccine for prevention of infection; thus, non-specific methods for prevention such as improvements in sanitation, provision of clean potable water and good food hygiene are important.[61]

Yersinia enterocolitica

The genus *Yersinia* comprises *Y. pestis*, the cause of plague, *Y. pseudotuberculosis* and *Y. enterocolitica*. Yersiniosis is a food-borne illness that has become more prevalent in recent years due to human transmission via the faecal–oral route and prevalence in farm animals. Yersiniosis is primarily caused by *Yersinia enterocolitica* and less frequently by *Yersinia pseudotuberculosis*.

Epidemiology

Although *Yersinia* infection is said to have a worldwide distribution, it is found much more commonly in temperate zones than in the tropics. Even in temperate countries, infection is more prevalent in colder climates and is more common in winter.[65] In most surveys of acute diarrhoeal disease where *Y. enterocolitica* was sought, it was either absent, or present in less than 1% of cases.[66] However, cases of generalized infection have been recorded in South Africa and other studies have shown infection in West Africa and Ethiopia.[67–69]

The reservoir for *Y. enterocolitica* is a variety of animal species, including birds, frogs, fish, snails, oysters and most mammals. The organism is excreted in faeces from pigs and cattle and can persist in lakes, streams, soil and vegetables. Patient-to-patient spread is rare except by blood transfusion. The incubation period is 1–11 days and bacteria are excreted for 14–97 (mean 42) days.

Bacteriology

Y. enterocolitica is a small Gram-negative rod with peritrichous flagella. It will grow on simple media and is lactose non-fermenting on MacConkey agar. It is psychrophilic and isolation from clinical samples often involves a cold enrichment step. O serogrouping is used to subdivide strains. The human pathogenic strains most frequently isolated worldwide belong to serogroups O:3, O:5,27, O:8 and O:9.

Pathogenesis

Pathogenic strains of *Y. enterocolitica* carry a large plasmid that encodes surface proteins and lipopolysaccharides mediating cell attachment, resistance to phagocytosis and serum resistance. *Yersinia* adhesion A protein (YadA) mediates mucus and epithelial cell attachment and, in concert with invasin, promotes host cell invasion. Induction of YadA expression is coordinated with the upregulation of Yops (*Yersinia* outer membrane proteins). Chromosomal genes (*inv*, *ail*: attaching invasion locus) encode the ability to invade epithelial cells. The Yops are translocated through a host-cell docked *Yersinia* secretion protein F needle, directly into the targeted host cells. The YopB and YopD proteins form a pore in the host cell plasma membrane, allowing for translocation of the effector proteins. YadA elicits an inflammatory response in epithelial cells by inducing mitogen-activated protein kinase-dependent IL-8 production and by contributing to the resulting intestinal inflammatory cascade.[70] Although *Y. enterocolitica* produces a toxin similar to LT, its role in pathogenesis is unclear. *Y. enterocolitica* invades ileal enterocytes and M cells in Peyer's patches, where it multiplies. This produces an inflammatory diarrhoea. Bacteria may pass to local lymph nodes and subsequently produce systemic disease.

In addition to disease produced directly by *Y. enterocolitica*, there are a number of autoimmune phenomena which present in a proportion of patients after initial infection. These include: erythema nodosum, reactive arthropathy, Reiter's syndrome and glomerulonephritis. In addition, there is a linkage with thyroid disorders, in that patients with Hashimoto's thyroiditis have high titres of *Y. enterocolitica*-agglutinating antibodies and that the surface of *Y. enterocolitica* has receptors for thyroid-stimulating hormone.

Clinical Features

Most symptomatic infections are in children under 5 years of age.[65] Characteristically, clinical features consist of diarrhoea, low-grade fever and abdominal pain. The diarrhoeic stool will be frankly blood-stained in a quarter of cases. Nausea, vomiting, headache and pharyngitis are minor presentations. The abdominal pain may be present alone or with mild diarrhoea and is often termed the pseudoappendicular syndrome. Infection may spread elsewhere to produce bacteraemia, peritonitis, hepatic, renal and splenic abscesses, pyomyositis and osteomyelitis.[65,68] These are more likely to occur in patients who are immunocompromised or who have iron overload – as in haemochromatosis.[67] The extraintestinal manifestations are more likely to occur in adults, as are the autoimmune phenomena. Of those with reactive arthritis, 80% are of HLA-B27 histocompatibility type.

Diagnosis

Y. enterocolitica can be isolated from stool, appendix, mesenteric lymph nodes, blood and other focal sites of infection, using simple media. Strategies for isolation include MacConkey agar incubated at 25–30°C for 48 hours or selective media such as cefsulodin–irgasan–novobiocin (CIN) agar at 37°C. For isolation from food or water, cold enrichment in phosphate-buffered saline for up to 4 weeks at 4°C prior to plating on to CIN agar greatly increases the yield of both pathogenic and non-pathogenic *Yersinia* spp. Speciation is obtained by biochemical tests and it is noteworthy that all non-pathogenic *Y. enterocolitica* have pyrizinamidase activity. Pathogenic *Y. enterocolitica* all possess the virulence plasmid. For retrospective diagnosis, serology using ELISA, whole cell agglutination, or complement fixation tests can be performed. They can be difficult to interpret and cross-reactions, for example *Y. enterocolitica* 0:9 with *Brucella abortus*, *E. coli*, *Morganella morganii* and *Salmonella* spp., do occur. The specificity of the test can be improved by detecting a greater than fourfold increase in titre between acute and convalescent sera.

Treatment and Control

Gastrointestinal infections are usually self-limiting and do not merit antimicrobial therapy. Nonetheless, fluoroquinolones or

Figure 24.7 Thin-section electron micrograph of *Clostridium perfringens* showing its endospore.

third-generation cephalosporins, the best therapeutic options, are warranted to treat enterocolitis in compromised hosts and in patients with septicaemia or invasive infection, in which the mortality can be as high as 50%.[71]

Clostridium spp.

Clostridia are anaerobic sporing Gram-positive rods (Figure 24.7). Two species, *Cl. perfringens* and *Cl. difficile*, are associated with diarrhoeal disease.

CLOSTRIDIUM PERFRINGENS

Two forms of diarrhoeal disease are associated with *Cl. perfringens* (formerly *welchii*). The first is a food-poisoning illness due to ingestion of *Cl. perfringens* type A or the enterotoxin it produces. Although this is a common cause of food poisoning in industrialized countries, it produces mild, short-lived disease and is extremely uncommon in the tropics. *Cl. perfringens* type C, in contrast, is common in certain areas of the tropics and produces severe necrotic enteritis.

Epidemiology

Cl. perfringens type C has been implicated in enteritis necroticans (Darmbrand) seen in malnourished individuals in Northern Europe after the Second World War and 'pigbel' in the highlands of Papua New Guinea. A similar disease has been described in Uganda, Malaysia, Thailand, Indonesia, China and, more recently, in India.[72–75]

Infection can occur sporadically but also in epidemics.[72–74] It occurs at any age but is more likely to present as acute toxic or acute surgical problems in children under 10 years old. In Papua New Guinea, pigbel is associated with large 'pig feasts' that occur every 3–10 years. Infection is more common in males than females; whether this represents a true difference in susceptibility is unclear. *Cl. perfringens* type C can be found in the human normal intestinal flora, in pig excreta and in soil.

Pathogenesis

The enteric toxins of *Cl. perfringens* share two common features: (1) they are all single polypeptides of modest (approx. 25–35 kDa) size, although lacking in sequence homology and (2) they generally act by forming pores or channels in plasma membranes of host cells. These enteric toxins include *Cl. perfringens* enterotoxin (CPE), which is responsible for the symptoms of a common human food poisoning and acts by forming pores after interacting with intestinal tight junction proteins. Two other *Cl. perfringens* enteric toxins, epsilon-toxin (a bioterrorism select agent) and beta-toxin, cause veterinary enterotoxaemias when absorbed from the intestines; beta- and epsilon-toxins then apparently act by forming oligomeric pores in intestinal or extra-intestinal target tissues. The action of a newly discovered *Cl. perfringens* enteric toxin, beta2 toxin, has not yet been defined but precedent suggests it might also be a pore-former.[76]

Since *Cl. perfringens* type C can be found as part of the normal intestinal flora, it is considered that host-dependent factors are also involved in pathogenesis. First, the bulk of the normal anaerobic flora is found in the large bowel and one hypothesis is that overgrowth of *Cl. perfringens* type C in the jejunum might be related to development of disease. A more attractive hypothesis links malnutrition and type of diet with disease. The β toxin is readily inactivated by intestinal proteases. Protein deficiency decreases intestinal protease levels; in addition, the sweet potato, which is a staple diet in highland Papua New Guinea, contains heat-stable trypsin inhibitors. Thus, consumption of meat contaminated by *Cl. perfringens* type C or its β toxin in an individual with low intestinal protease activity due to malnutrition or dietary protease inhibitors would allow the toxin to produce intestinal damage.[74,76]

Pathology

Gross pathology shows patchy segmental acute ulcerative necrosis of the jejunum and, to a lesser extent, the ileum, caecum and colon. This may rapidly progress to segmental gangrene with gas in the mucosa, mesentery or lymph nodes. Microscopically, the intestinal wall shows separation of the mucosa from the submucosa, with large denuded areas covered with a pseudomembrane of dead enterocytes and infiltrating neutrophils and red blood cells. Healing occurs with fibrosis and strictures and adhesions may form later.

Clinical Features

Pigbel varies in severity from mild diarrhoea to a rapidly fatal necrotizing enteritis, with high mortality (up to 85%). The incubation period is approximately 48 hours after the feast but can vary from 24 hours to up to a week.

Disease has been classified into four main presentations.[72] Type I (acute toxic) presents with fulminant toxaemia and shock. Type II (acute surgical) presents as mechanical and paralytic ileus, acute strangulation, perforation and peritonitis. Type III (subacute surgical) presents later, with complications of mild type II. Finally, type IV (mild or trivial) presents with mild diarrhoea but may rarely progress to type III. Type I disease occurs most commonly in young children and has the highest mortality (85%). Type II disease has a 42% mortality; type III, 44% mortality; and type IV is never fatal. In type II and type III disease, a palpable segment of thickened intestine may be found. The stool will contain blood and pus cells and there is a neutrophil leukocytosis in peripheral blood. The differential diagnosis includes: acute causes of inflammatory diarrhoea, peritonitis, acute abdominal obstruction, acute pancreatitis, acute amoebic colitis and sickle cell crises.

Diagnosis

Cl. perfringens can be cultured from faeces, peritoneal fluid or other infected sites by plating on to neomycin blood agar and incubating anaerobically. *Cl. perfringens* type C is differentiated from other *Cl. perfringens* by serological techniques, including immunofluorescence and type C antibody-coated silica beads.[77] Interpretation of results can be difficult since *Cl. perfringens* type C is also found in normal individuals. Detection of antibodies to the toxin can be useful in reaching a diagnosis in survivors.

Treatment

Acute resuscitation is by fluid and electrolytes intravenously, together with bowel decompression by restricting oral intake and nasogastric intubation. Antibiotics will be needed if there is extraintestinal spread of the organism (e.g. peritonitis) and metronidazole, ampicillin, chloramphenicol or penicillin should be of value. Administration of *Cl. perfringens* type C antiserum is also beneficial. Surgical intervention will be necessary if there is persisting obstruction, increasing signs of toxaemia, or signs of peritonitis or of strangulation. There is some evidence that early surgical intervention can decrease mortality.

Prevention

Active immunization with a toxoid prepared from *Cl. perfringens* type C toxins has decreased the incidence of pigbel in children in the past.[78]

CLOSTRIDIUM DIFFICILE

Cl. difficile is the cause of antibiotic-associated colitis and of pseudomembranous colitis. In adults, a clinical prediction rule found the best signs to be: significant diarrhoea ('new onset of more than three partially formed or watery stools per 24 hour period'), recent antibiotic exposure, abdominal pain, fever (up to 40.5°C) and a distinctive foul stool odour. The bacteria release toxins that can cause bloating and diarrhoea, with abdominal pain, which may become severe. Symptoms of *Cl. difficile* infection often mimic some flu-like symptoms and can mimic disease flare in patients with inflammatory bowel disease-associated colitis. *Cl. difficile* infections (CDI) are the most common cause of pseudomembranous colitis and in rare cases this can progress to toxic megacolon, which can be life-threatening. The bacterium can be found worldwide but its role as a cause of diarrhoeal disease in developing countries is probably underestimated. The new hypertoxin-producing BI/NAP1/O27 strain of *Cl. difficile* is thus far confined to developed countries.[79] Of all patients treated for CDI, 20% relapse and 65% of those experiencing a second relapse become chronic cases.

Pathogenic *Cl. difficile* strains produce several known toxins. The most well-characterized are enterotoxin (*Cl. difficile* toxin A) and cytotoxin (*Cl. difficile* toxin B), both of which are responsible for the diarrhoea and inflammation seen in infected patients, although their relative contributions have been debated. Toxins A and B are glucosyltransferases that target and inactivate the Rho family of GTPases. Toxin B (cytotoxin) induces actin depolymerization by a mechanism correlated with a decrease in the ADP-ribosylation of the low molecular mass GTP-binding Rho proteins. Another toxin, binary toxin, has also been described, but its role in disease is not yet fully understood.[80]

Current recommendations include metronidazole for treatment of mild to moderate CDI and vancomycin for severe CDI. Results from small clinical trials suggest that nitazoxanide and teicoplanin may be alternative options to standard therapies, whereas rifaximin has demonstrated success in uncontrolled trials for the management of multiple recurrences.[81] Anecdotal reports have also suggested that tigecycline might be useful as an adjunctive agent for the treatment of severe complicated CDI. Fidaxomicin, a macrocyclic antibiotic, has a narrow spectrum of activity against Gram-positive anaerobes and is bactericidal against *Cl. difficile*. It has no activity against Gram-negative bacteria. Fidaxomicin has minimal activity against *Bacteroides* species, which may be advantageous in maintaining colonization resistance and protecting the gastrointestinal tract from colonization by *Cl. difficile*.[82] A vaccine is being developed targeted at closed communities, hospitals and those patients needing prolonged antibiotic therapy.[83]

The cornerstone of prevention is appropriate contact precautions and strict hand hygiene. Other important approaches are effective cleaning and antibiotic stewardship.[84]

Aeromonas and *Plesiomonas*

These two genera within the Vibrionaceae family are both aquatic microorganisms and can be readily isolated from fresh and salt water, fish, soil and food. Today, the genus *Aeromonas* is regarded not only as an important disease-causing pathogen of fish and other cold-blooded species but also as the aetiological agent responsible for a variety of infectious complications in both immunocompetent and immunocompromised persons.[85]

AEROMONAS HYDROPHILA

Epidemiology

A. hydrophila has been associated with gastroenteritis in many countries throughout the world. In tropical countries, it can be isolated from healthy as well as diarrhoeic individuals. In Thailand, *Aeromonas* spp. were isolated from 9% of cases of gastroenteritis and were second in importance only to ETEC.

Microbiology

The genus *Aeromonas* encompasses three motile species which cause disease in humans: *A. hydrophila*, *A. caviae* and *A. sobria*. A fourth, non-motile species, *A. salmonicida*, is a fish pathogen and will not grow above 30°C. They are oxidase-positive and will grow on most simple media. *Aeromonas* produces a wide range of extracellular factors including: proteases, elastases, esterases, DNAse, haemolysins, cytotoxins and enterotoxins.

Pathogenesis

Aeromonas is associated with both inflammatory and non-inflammatory diarrhoea. It possesses both fimbrial and non-fimbrial adhesins for attachment to the intestinal mucosa. *Aeromonas* produces two types of flagella, a constitutively expressed polar flagellum (Pof) and multiple inducible lateral flagella (Laf). Pof produces swimmer cells in liquid environments, while Laf induces swarming motility on solid medium surfaces. *Aeromonas* produces biofilms, which are regulated by

TABLE 24.3 Classification of *Shigella* Serotypes

Species	No. of Serotypes	Glucose	Mannitol (Fermentation)	Lactose
Sh. dysenteriae	10	+	–	–
Sh. flexneri	6	+	+	–
Sh. boydii	15	+	+	–
Sh. sonnei	1	+	+	Late

quorum sensing. Once established in the gastrointestinal tract, aeromonads can apparently produce diarrhoea by elaboration of enterotoxigenic molecules, causing enteritis, or by invasion of the gastrointestinal epithelium, producing dysentery or colitis.

Clinical Features

Based upon frequency, *Aeromonas* clinical infections fall into four broad categories, namely: (1) gastrointestinal tract syndromes, (2) wound and soft tissue infections, (3) blood-borne dyscrasias and (4) a miscellaneous category which includes a myriad of less frequently encountered ailments and infectious processes. Gastroenteritis associated with *Aeromonas* spp. can vary from acute watery diarrhoea with fever to chronic dysentery with fever and abdominal cramps.[85]

Diagnosis

Aeromonas can be isolated from faeces using selective media such as ampicillin blood agar. Prior enrichment in alkaline peptone water increases the sensitivity of isolation. Since *Aeromonas* spp. can be isolated from normal individuals, isolation does not prove causation. For the future, it may be necessary to detect pathogenicity factors (toxins, adhesins or invasiveness) to link isolation with the disease in a particular patient.

Treatment

Rehydration is usually the only intervention needed. If infection becomes disseminated or there is chronic dysentery, antimicrobials such as fluoroquinolones might be of benefit.

PLESIOMONAS SHIGELLOIDES

This bacterium has been associated with food-borne (usually fish) gastroenteritis in Mali and India and there has even been a case of snake-to-human transmission.[86–88]

Shigellosis (Bacillary Dysentery)

Dysentery has been a disease of poor and crowded communities throughout history and continues to be a major cause of morbidity and mortality in the tropics. Dysentery bacilli were first demonstrated by Shiga in 1898 and subsequent studies showed that four species, *Shigella dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei*, were responsible for the disease described as bacillary dysentery. *Sh. dysenteriae* and *Sh. flexneri* are responsible for most infections in the tropics. A recent review of the literature for 1990–2009 indicates that ~125 million shigellosis cases occur annually in Asia, of which ~14 000 are fatal.[89] Shigellosis occurs both endemically and as epidemics.

Bacteriology

Shigella species are members of the Enterobacteriaceae and are aerobic, Gram-negative, non-motile bacilli. In pure growth, *Shigella* spp. are readily cultured on non-selective media, but for isolation from clinical specimens, selective media such as MacConkey and xylose lysine deoxycholate (XLD) are necessary. They are typically non-lactose-fermenting, lysine-decarboxylase-negative and do not produce gas from glucose. The exceptions are *Sh. sonnei*, which ferments lactose slowly and *Sh. flexneri* 6 and *Sh. boydii* 13, which produce gas from glucose. *Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii* are each divided into a number of serotypes (Table 24.3).

Serotype (O) antigens are located on the outer polysaccharide chains of the lipopolysaccharide component of the cell wall. *Shigella* spp. are non-motile and do not possess H antigens. For epidemiological studies, serotypes may be subdivided by molecular methods such as the enterobacterial repetitive intergenic sequence-based polymerase chain reaction (ERIC-PCR) typing or the current standard for the typing of Shigella, pulsed field gel electrophoresis (PFGE).[90]

Pathogenesis

Shigella dysentery is characterized by invasion of the colonic mucosa, local spread of the infecting organism and death of intestinal epithelial cells. In a proportion of cases, extraintestinal complications occur, including seizures, hyponatraemia and hypoglycaemia, septicaemia, Reiter syndrome, encephalopathy and the haemolytic–uraemic syndrome. Interaction of *Shigella* with epithelial cells includes contact of bacteria with the cell surface and release of Ipa proteins through a specialized type III secretion. A complex signaling process involving activation of small GTPases of the Rho family and c-src causes major rearrangements of the cytoskeleton, thereby allowing bacterial entry by macropinocytosis. After entry, shigellae escape to the cell cytoplasm and initiate intracytoplasmic movement through assembly of actin filaments caused by bacterial surface protein IcsA, which binds and activates a protein inducing actin nucleation. Actin-driven motility promotes efficient colonization of the host cell cytoplasm and rapid cell-to-cell spread via protrusions that are engulfed by adjacent cells in a cadherin-dependent process. Bacterial invasion turns infected cells to strongly pro-inflammatory cells through sustained activation of nuclear factor-kappaB. A major consequence is interleukin (IL)-8 production, which attracts polymorphonuclear leukocytes (PMNs). On transmigration, PMNs disrupt the permeability of this epithelium and promote its invasion by shigellae. At the early stage of infection, M cells of the follicle-associated epithelium allow bacterial translocation. Subsequent apoptotic killing of macrophages in a caspase 1-dependent process causes the release of IL-1β and IL-18, which accounts for the initial steps of inflammation.[91] A number of pathogenic factors and their genetic determinants have been described. Invasion is associated with specific outer membrane proteins that are encoded on a plasmid (extrachromosomal) DNA of size 220 kb. Strains not containing these plasmids have been shown to be non-virulent. *Sh. dysenteriae* 1 produces a toxin, Shiga toxin (Stx). Stx inactivates

ribosomal RNA, inhibiting protein synthesis and leads to cell death. Stx is composed of A and B subunits, the genes being chromosomally located and is the same as SLT1 and SLT2 produced by EHEC. The cytotoxic effects of *Shiga* toxin are involved both in the haemorrhagic intestinal manifestations and in the haemolytic–uraemic syndrome.

Pathology and Immunology

The characteristic pathology is an acute, locally invasive colitis, ranging from mild inflammation of the mucous membranes of the distal colon to severe necrosis of much of the large bowel. Sigmoidoscopy reveals a red, bleeding mucosa with patches of necrotic membrane, which may separate to leave ulcerated areas. The inflammatory process may extend through the submucosa to the muscle layer. In severe cases, complete healing may not occur, resulting in fibrous tissue formation and persistent ulceration. Bacteraemia is uncommon in *Shigella* infection, but is a probable risk factor for increased mortality.[92] Circulating endotoxin is likely to play an important role in the systemic manifestations of *Shigella* infection. In *Sh. dysenteriae* 1 infections, Shiga toxin exerts both enterotoxic effects, through specific glycolipid binding sites and is responsible for the haemolytic–uraemic syndrome. Infection with *Shigella* spp. leads to both local (gut) immunity and the production of circulating antibodies. Circulating antibodies are directed against the O (lipopolysaccharide) antigens and have been shown to be serotype-specific.

Epidemiology

Man is the only natural host for infection by *Shigella* spp. Infection is by ingestion, the infective dose being as low as 10–100 bacteria for *Sh. dysenteriae*. The incubation period is 1–5 days. Shigellosis occurs as an endemic disease in conditions of crowding, poor sanitation and inadequate water supply and is primarily a disease of poor disadvantaged communities in the tropics.

Endemic shigellosis is largely a paediatric disease, most cases occurring in children below 10 years of age. In a recent review of data from Asia, the median frequency of *Shigella* spp. isolation from diarrhoea cases in the community in children 0–4 years of age was 4.4%, while at facilities it was 6.6% and in older individuals it was 4.0% in the community and ~11.6% in facilities.[90] Routes of infection include direct person-to-person transmission (from cases or asymptomatic excreters) and transmission via contaminated water or food. The evidence for person-to-person transmission in endemic areas of the tropics comes from a number of community studies that show a high frequency of secondary household cases in the family of an index case, but no differences between families with cases and control families in relation to water or food supply.[93] In epidemics of *Sh. dysenteriae* 1, person-to-person transmission is also more common than point-source food or water outbreaks. Though occasional water-borne epidemics have been described, a seasonal pattern of shigellosis is seen in most endemic areas. In Bangladesh, peak transmission rates occur at the beginning of the monsoon season, with a second, lower peak in the winter season.[94]

Clinical Features

Shigellosis may vary from relatively mild watery diarrhoea to severe dysentery with intestinal and extraintestinal complications. In severe cases, the onset is abrupt, with tenesmus, fever and frequent passage of bloody, mucoid stools. The degree of dehydration may be considerably less than in other diarrhoeas, though stool frequency may be as many as 30 times per day. Diarrhoea is often accompanied by fever, headache and malaise. Intestinal complications include toxic megacolon, perforation and a protein-losing enteropathy. Electrolyte imbalance may arise – in particular, prolonged hyponatraemia. *Sh. dysenteriae* and *Sh. flexneri* infections may result in a number of extraintestinal complications. Haemolytic–uraemic syndrome occurs particularly with *Sh. dysenteriae* 1 and can develop 7–10 days after the onset of disease. Convulsions may occur with infections caused by all species of *Shigella*, particularly in children. They may occur before diarrhoea begins and are usually accompanied by a rising fever. Encephalopathy and hemiplegia have been reported.[95]

Diagnosis

In many parts of the tropical world, the diagnosis and subsequent management of *Shigella* infections occur in the absence of laboratory facilities. While clinical algorithms (Figure 24.8) have been used to aid in the differential diagnosis of dysentery symptoms, the more general case definition of 'acute diarrhoea with visible blood in the stools' is the clinical case definition recommended for surveillance.[96] Laboratory isolation and identification of *Shigella* spp. is necessary to confirm the

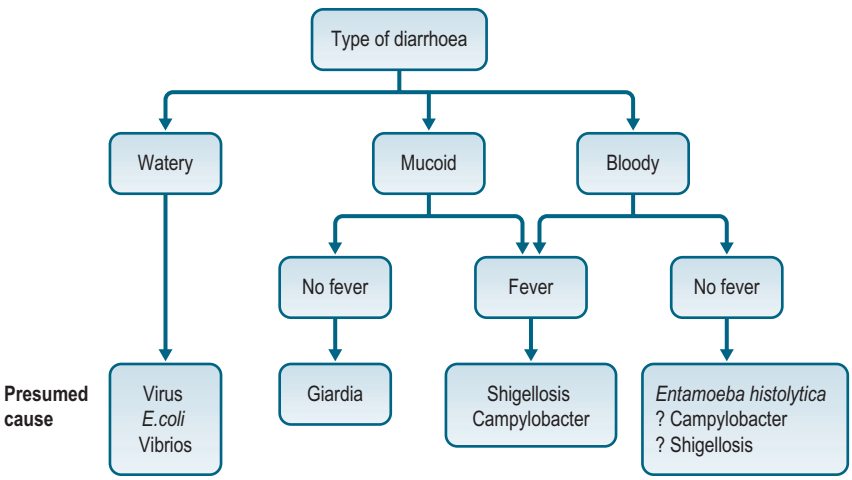

Figure 24.8 Clinical algorithm in the differential diagnosis of diarrhoea.

diagnosis and to enable antimicrobial sensitivities to be determined.

Shigellae survive poorly in ambient temperatures in the tropics and if the stool specimens cannot be cultured within a few hours of collection, they should be placed in transport medium and stored at +4°C. Cary–Blair medium and buffered glycerol saline (BGS) are the recommended transport media. In the investigation of epidemics, it is more useful to collect specimens from a small number of patients who fit the clinical case definition and to ensure that these specimens are transported and processed appropriately. For molecular approaches, new transport technique (DNA/RNA Protect™, Sierra Diagnostics Inc., Sonora, CA, USA) permits detection of *ipaH* from stool specimens held for prolonged periods at room temperature.[97]

Figure 24.9 shows the WHO guidelines for the culture of specimens for isolation and identification of *Shigella* spp. Faecal specimens or rectal swabs should be cultured overnight on MacConkey medium and a more selective medium such as xylose lysine deoxycholate (XLD) agar. Shigellae appear as pale, non-lactose-fermenting colonies on MacConkey medium and as pink colonies on XLD. Suspect colonies are incubated overnight on Kligler iron agar (KIA) and motility indole urea (MIU) medium. Table 24.4 shows the typical reactions of *Shigella* spp. in these composite media. Positive isolates may be typed by slide agglutination using the appropriate *Shigella* antisera. Antimicrobial sensitivities should be determined using a disc diffusion method. It is essential that a standardized technique is used and the Kirby Bauer-based CSLI (formerly NCCLS) methodology is recommended.[98]

Management

The management of cases of shigellosis requires appropriate rehydration and electrolyte therapy, antimicrobial treatment and the management of complications. Dehydration is rarely severe; oral rehydration is usually sufficient to restore water and electrolyte imbalances. High-risk patients include children less than 5 years of age, patients who are dehydrated or seriously ill when first seen and older children and adults who are malnourished. Effective antimicrobial therapy will shorten the duration of illness and is particularly necessary in severe cases. Resistance of *Shigella* spp. to commonly used antimicrobial agents is an increasing problem in many tropical countries and data on local sensitivities are essential if effective treatment is to be implemented.

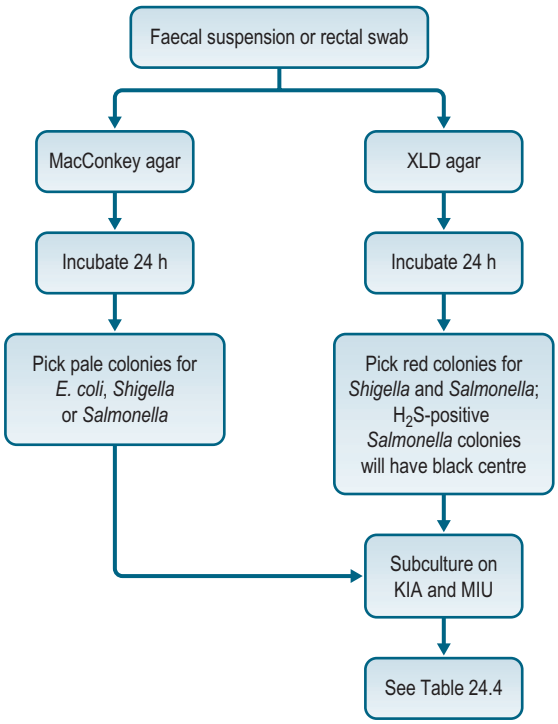

Figure 24.9 Culture protocol for isolation of *Shigella* spp.

TABLE 24.4 Reactions of *Shigella* spp. on Kligler Iron Agar (KIA) and Motility Indole Urea (MIU) Medium

Bacterium	Urea	Slant	Butt	H_2S	Gas	Motility	Indole
E. coli	–	A	A	–	+	(+)	D
Sh. dysenteriae	–	K	A	–	–	–	D
Sh. flexneri	–	K	A	–	–[a]	–	D
Sh. boydii	–	K	A	–	–[b]	–	D
Sh. sonnei	–	K	A	–	–	–	–

A, acid (yellow) reaction; K, alkaline (red) reaction; +, positive reaction; –, negative reaction; d, different biochemical types.
[a]Some *Sh. flexneri* serotype 6 gas (+).
[b]Serotypes 13 and 14 gas (+).

Resistance of *Sh. dysenteriae* to ampicillin, co-trimoxazole and chloramphenicol is now widespread and nalidixic acid is the first-line drug of choice where resistance is not wide spread. Resistance to nalidixic acid is increasing, leaving only fluoroquinolones such as ciprofloxacin and ofloxacin and pivmecillinam as effective oral therapies. Several clinical trials have demonstrated the efficacy of short courses of fluoroquinolones being effective.[99] Unresolved issues remain over the use of fluoroquinolones in children. The importance of local sensitivity data cannot be over-emphasized.

Maintaining adequate levels of nutrition in patients is an essential component of management, particularly in children, who may already be malnourished. Studies in Bangladesh have shown the value of supportive nutrition in the outcome of children with shigellosis.

Prevention and Control

Shigellosis is primarily a disease of crowded and usually poor communities, living in an environment characterized by inadequate sanitation and often polluted water. In the long term, the incidence of shigellosis will be reduced only by improved public health and the alleviation of poverty. Since most transmission is from person to person, improvements in water supply quality alone may have little impact. Most studies show that increased water quantity and general improvement in the level of hygiene reduce the incidence of diarrhoeal disease. Improvement in hygiene at the household level, particularly through the provision of soap for hand washing, has been shown to reduce the transmission of shigellosis. In epidemics of shigellosis, coordinated action is necessary at the local and regional level in diagnosis, local public health interventions and possibly restrictions on population movements, markets, religious gatherings, etc.

There are no effective vaccines against shigellosis, although current approaches include (1) live attenuated deletion mutants based on rational selection of genes that are key in the

TABLE 24.5 The First Six Cholera Pandemics

Pandemic	Date	Indian Sub-Continent	South-East Asia	Middle East	Europe	North Africa	East Africa	America
First	1817–1823	+	+	+	–	–	+	–
Second	1826–1837	+	+	+	+	+	+	+
Third	1842–1862	+	+	+	+	+	+	+
Fourth	1865–1875	+	+	+	+	+	+	+
Fifth	1881–1896	+	+	+	+	+	+	+
Sixth	1899–1923	+	+	+	+	–	+	–

pathogenic process and (2) conjugated detoxified polysaccharide parenteral vaccines, or more recently conjugated synthetic carbohydrates. Some of these approaches have already undergone phase I and II clinical trials with promising results, but important issues have also emerged, particularly the discrepancy between colonization and immunogenic potential of live attenuated vaccine candidates depending upon the population concerned, particularly in endemic areas.[100] For the foreseeable future, however, prevention of morbidity and mortality caused by shigellosis will depend on public health interventions and effective and timely case management.

Vibrio cholerae

Cholera occurs endemically in many areas of the tropics, particularly in South and South-east Asia and Africa. In 1991, cholera appeared in Latin America for the first time in the twentieth century. A cholera-like disease was described by early Indian, Greek and Chinese writers, but it is uncertain whether the disease had spread beyond the Indian subcontinent before the nineteenth century. From 1817 to 1923 there were six pandemics of cholera, spreading extensively from its natural home in the Gangetic plain and delta (Table 24.5). The seventh pandemic of cholera, which began in 1961, is described under Epidemiology (see below).

Bacteriology

In 1883, Koch demonstrated the bacterial cause of cholera during a visit to Egypt and subsequent work defined the species *Vibrio cholerae*. Vibrios are comma-shaped, aerobic Gram-negative bacteria which have a characteristic darting movement (Figure 24.10). They are oxidase-positive and ferment sucrose and glucose but not lactose. Vibrios possess both flagellar and somatic antigens. The species *V. cholerae* is divided into many serovars, according to somatic antigens. Until the appearance of *V. cholerae* serotype O139 in 1992, *V. cholerae* O1 was the only serotype responsible for cholera. Other serovars with different O antigens may cause a diarrhoea-like illness but are not associated with epidemic cholera. There are two biotypes of *V. cholerae* O1: classical and El Tor. Table 24.6 summarizes their characteristic properties. *V. cholerae* El Tor was first isolated from pilgrims at the El Tor quarantine station in Sinai in 1906. Until 1961, the El Tor biotype was isolated only in Sulawesi, Indonesia and had caused four localized epidemics between 1937 and 1958. The classical and El Tor biotypes are each divided into three serotypes: Ogawa, Inaba and Hikojima. *V. cholerae* O139 is related to the El Tor biotype.

V. cholerae does not form spores and is killed by heating at 55°C for 15 minutes and by phenolic and hypochlorite disinfectants. It can survive in saline conditions at low temperatures

TABLE 24.6 Differentiating Properties of Classical and El Tor Biotypes of *Vibrio Cholerae* 01

	Classical	El Tor
Chicken cell haemagglutination	–	+
Voges–Proskauer test	–	+
Polymyxin B sensitivity	Sensitive	Resistant

for up to 60 days and may survive in aquatic environments for extended periods in a non-cultivable state. *Vibrio cholerae* is often associated with zooplankton and shellfish in water and it can use chitin as a carbon and nitrogen source. In water, *V. cholerae* enter a viable but non-culturable form, also called active but non-culturable or conditionally viable environmental cells.[101] Excluding seafoods, *V. cholerae* survives for only a limited time on foodstuff, although contaminated food may act as a vehicle for transmission. In fish and shellfish, *V. cholerae* may survive from 2 to 5 days at ambient temperatures, a property often associated with food-related outbreaks.

Pathogenesis and Immunity

Cholera is characterized by severe watery diarrhoea leading to dehydration, electrolyte imbalance and hypovolaemia, with a mortality ranging from less than 1 to 40%.

There is a wide spectrum of severity and mild and asymptomatic cases may occur. *V. cholerae* is non-invasive;

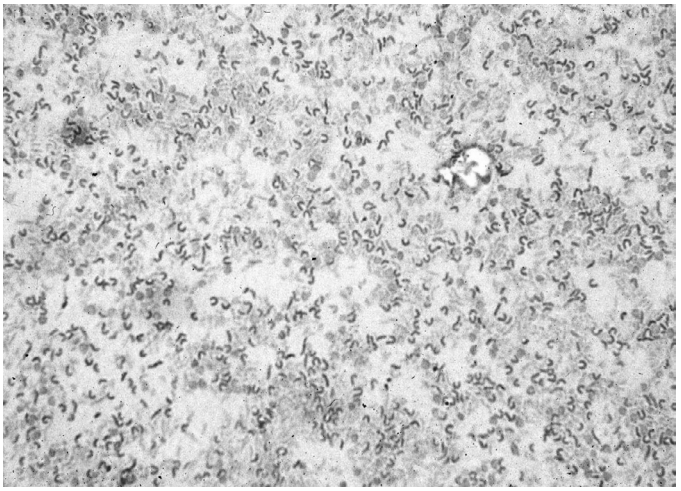

Figure 24.10 Gram stain of *V. cholerae* showing typical comma-shaped bacilli.

pathogenesis is due to an enterotoxin that causes excessive fluid and electrolyte loss. Bacteria that survive gastric acid reach the small intestine and elaborate cholera toxin, the major virulence factor for pathogenic strains. Cholera toxin consists of one A subunit associated with five B subunits. The B subunit pentamer binds to the ganglioside GM1 on eukaryotic cells and the A subunit is translocated intracellularly, where it activates adenylate cyclase and raises intracellular cyclic AMP. This leads to chloride secretion through the apical chloride channel and secretory diarrhoea. The second major virulence factor of pathogenic strains of *V. cholerae* is the toxin-coregulated pilus, a colonization factor whose expression is regulated in parallel to cholera toxin.

The genes for cholera toxin are encoded within the genome of a filamentous bacteriophage, the CTX phage. The classical and El Tor strains have different versions of this bacteriophage, which can insert at one or two attachment sites in the genome depending on the biotype. The bacterial cell surface receptor for CTX phage is the toxin-coregulated pilus, which is encoded within the vibrio pathogenicity island (VPI-1). Virulence in *V. cholerae* evolved with sequential acquisition of VPI-1 followed by the CTX phage and all pandemic strains of El Tor have two vibrio pathogenicity islands.

VPI-1 and VPI-2 are additional toxins and factors known to be involved in cholera pathogenesis include Zonula occludens toxin (Zot), which increases the permeability of the small-intestinal mucosa by affecting the structure of the intercellular tight junctions and the accessory cholera exotoxin (Ace) which increases transmembrane ion transport.

Immunity to both cholera toxin and bacterial surface antigens follows natural infection. Most studies of immune response have measured serum vibriocidal antibodies which have been shown to be mainly against LPS antigens. In a study of cholera index patients and their household contacts containing both O1 or O139 index cases, it was shown that circulating IgA levels for anti-LPS, anti-TcpA (TcpA being the major TCP pilus subunit) and anti-cholera toxin B-subunit (CTB) can be correlated with protection from O1 infection and that circulating anti-TcpA IgA correlates with protection from O139, whereas circulating IgG showed no such correlations.[102] Anti-TcpA responses could, therefore, be helpful to the generation of cross-O1/O139 protection and IgA may be important to protection from natural cholera infection.

Epidemiology

Man is the only known natural host of *V. cholerae*. Transmission is by ingestion, through contaminated water or food. The infective dose is high, up to 10^{11} bacteria being required. The incubation period ranges from a few hours to 5 days.

Serological studies have shown that, both in endemic areas and during outbreaks, for each symptomatic case there may be from 5 to 40 infected but asymptomatic or mildly symptomatic cases. Contamination of water or food may thus occur from symptomatic cases or asymptomatic, transient carriers. Most are free from infection within 2–3 weeks and there have been few examples of persistent carriage.

V. cholerae O1 can survive for weeks to months in the natural aquatic environment, but there is uncertainty whether this occurs only in relation to frequent contamination by infected persons, or whether *V. cholerae* O1 truly survives as an environmental bacterium. Studies have described the occurrence of 'non-culturable' dormant strains, which may persist for long periods in natural aquatic environments.[101] Change from the dormant to a culturable form may be influenced by environmental factors influencing toxin regulatory genes.

There are important epidemiological differences between classical and El Tor *V. cholerae*. For El Tor, the ratio of carriers to cases may range from 30:1 to 50:1, compared with 5:1 for classical. El Tor can also survive for longer periods in the environment. These factors give El Tor an epidemiological advantage in the spread of the disease, which has occurred in the seventh pandemic and has contributed to the displacement of the classical type by El Tor. Only in parts of southern Bangladesh has the classical biotype persisted beyond the 1980s.[103]

The seventh pandemic of cholera began in 1961, originating on the island of Sulawesi in Indonesia. The pandemic strain was *V. cholerae* O1 El Tor and it spread rapidly to countries of Southeast Asia. Between 1963 and 1969 the pandemic spread to the Indian subcontinent, displacing the classical biotype and by 1970 had reached the Middle East. The pandemic entered Africa by two routes, in West Africa, probably by a returning traveller and from the Arabian peninsula, through Djibouti into East Africa. By 1978, most countries of central and southern Africa were affected. The final stage of the pandemic was the arrival of cholera in the South American continent in January 1991, the first time that cholera had entered the continent since the fifth pandemic in the 1880s.

V. cholerae O139 was first isolated in south India in 1992. It was designated O139 as it did not agglutinate with O1 antisera, nor with antisera to any of the 137 other known, non-cholera-producing serotypes of *V. cholerae*. During 1992–1994, *V. cholerae* O139 spread to Bangladesh, where for some time it was the dominant serotype.[101] In the past decade, devastating epidemics of cholera have occurred in Angola, Ethiopia, Zimbabwe, Pakistan, Somalia, Sudan, Vietnam and Haiti.[104] In Haiti, following the earthquake, cholera appeared in 2010, affecting over 275 000 individuals with over 2000 fatalities. Genomic sequence analysis identified the source of the strain as a UN contingent from South Asia.[105]

Recent seventh pandemic strains have been described that have the classical CTX phage instead of the El Tor CTX phage, or a variant of the El Tor CTX phage encoding the B subunit of cholera toxin that occurs in classical V cholerae O1 strains.[106] These variant El Tor strains have largely replaced the earlier El Tor strains and might be associated with more severe diarrhoea.

Clinical Features

The clinical picture of infection with *V. cholerae* O1 may range from mild diarrhoea to severe dehydration with death occurring within hours. In most cases there is progress from the onset of diarrhoea to shock in 4–12 hours, with death following in several days if adequate management is not instituted. The symptoms are a reflection of the severe dehydration, electrolyte loss and metabolic acidosis. Hypovolaemia and hypotension lead to impaired consciousness and to renal failure. Hypoglycaemia may occur, particularly in children. Electrolyte loss leads to hyponatraemia and hypokalaemia. The latter may result in ileus, muscle weakness and cardiac arrhythmias.

Diagnosis

In epidemics, the diagnosis of cholera may be made presumptively on clinical and epidemiological grounds. The WHO clinical case definition for suspected cholera, or 'acute watery

diarrhoea', is 'a patient 5 years of age or older, who develops acute watery diarrhoea with or without vomiting', with the caveat that it is in an area where cholera is likely to occur. Laboratory diagnosis may be required when sporadic cases occur and when an extensive outbreak requires confirmation and typing of the aetiological agent. Dark-field microscopy of faecal specimens may show the characteristic darting movement of the vibrios. Inhibition of movement by addition of diluted O1 antisera to the slide will provide strong evidence that *V. cholerae* O1 is the causative agent. To confirm the diagnosis, specimens need to be cultured on a selective medium, such as thiosulphate citrate bile salt sucrose (TCBS) agar. Specimens should be transported from the field in alkaline peptone water or Cary–Blair transport medium and kept cool. *V. cholerae* O1 yields yellow, oxidase-positive colonies after overnight incubation on TCBS, which may be confirmed by slide agglutination with specific antiserum. In outbreak investigations, isolates should be sent to a reference laboratory for biotyping and serotyping. Sensitivity to tetracycline and other antimicrobial agents should be performed on a selected number of isolates. Where detailed epidemiological data are required, molecular methods have been used to distinguish different strains.[106]

Case Management

The successful management of cholera cases relies on adequate and appropriate rehydration and restoration of electrolyte balance. Except in the most severe cases, oral replacement solutions may be used. Oral solutions are based on the role of glucose enhancing the active uptake of sodium and water. As glucose is rarely available in rural areas, sucrose and rice-water-based solutions have been used with success.[105] The volume of replacement will depend on the degree of dehydration and the rate of continuing fluid loss. The compositions of oral and intravenous rehydration solutions are given in Table 24.7. WHO guidelines provide detailed protocols for rehydration and fluid maintenance.[107] Severe dehydration is characterized by 10% loss of body weight, lethargy or impaired consciousness, hypovolaemic shock and acidosis. In such patients, rapid intravenous rehydration is necessary, using a large-bore needle and multiple sites if necessary, aiming to restore normal hydration and acid–base balance within 2–3 hours. Of the losses, 50% should be replaced in the first 30–45 min, at a rate of 30 mL/kg, requiring 1–2 L in adults. Rehydration should then be slowed to 1 L per 30–45 min until normal hydration is achieved. Once rehydration is achieved, the maintenance phase requires the replacement of continuing stool losses. In the severely ill patient, this may require continuing intravenous therapy for some time, but in most cases oral rehydration using WHO or other rehydration solution is appropriate. Fluid replacement should be in the ratio of 1.5 volumes of oral fluid for each volume of stool. For children, this will be 100–200 mL per stool passed. In adults, in the recovery stage, fluid can be given as required. Moderate dehydration, characterized by 5% loss of body weight, clinical dehydration (poor skin turgor etc.) but no acidosis or shock, requires oral or intravenous rehydration initially, followed by oral maintenance. In adults, 2–4 L of ORS may be required in the first 4 hours to ensure rehydration. Potential complications in severely ill patients on presentation and during intravenous therapy include renal failure, hypoglycaemia, particularly in children and in prolonged dehydration, hypokalaemia and ileus and pulmonary oedema during rapid intravenous therapy when the metabolic acidosis has not been corrected, which is more likely when normal saline alone is used for rehydration. Hypokalaemia may occur during the maintenance phase, but should be uncommon if potassium-containing oral fluids are used.

Antimicrobial agents have been shown to shorten the period of diarrhoea and the amount of fluid loss. Tetracycline and doxycycline are the drugs of choice in adults where strains are sensitive, but the increasing occurrence of resistant strains limits their usefulness. Co-trimoxazole and furazolidone have been used, but antibiotics are secondary to the importance of early rehydration. Single-dose azithromycin is the preferred therapy in children.[105]

TABLE 24.7 Composition of Rehydration Solutions

Solution	Composition (mmol/L)				
	Na	Cl	K	Bicarbonate	Glucose
Ringers	130	109	4	28	0
Dhaka	133	98	13	48	0
WHO ORS	90	80	20	30	111
Reduced osmolarity ORS	50	40	20	30 citrate	111

Prevention and Control

Cholera is transmitted by the faecal–oral route through the contamination of water or food. Hence, public health measures to improve water and sanitation are essential for long-term control. The management of outbreaks is based on interrupting transmission, appropriate control and management of cases and contacts and effective surveillance. In most cholera outbreaks the source and routes of transmission are not obvious and general sanitary measures will need to be imposed. These may include chlorination of water supplies, boiling of water at household level and construction and maintenance of temporary latrines. Action will need to be taken to control the cleanliness of markets and the postponement of festivals and gatherings. Adequate, though basic, sanitation facilities must be made for disposal of faeces from cases during treatment.

The most appropriate group for chemoprophylaxis is household contacts of cases. The relatively high carriage rate of *V. cholerae* in this group has been described previously. Assuming strains are sensitive, tetracycline or doxycycline may be used in adults. For doxycycline, a single oral dose of 300 mg is adequate. The formerly used killed whole cell vaccines, given parenterally, have no useful role to play in the management or prevention of cholera: individual protection does not exceed 50–60%; vaccination does not reduce excretion of vibrios and is likely to give a false sense of security to both the affected population and the authorities during outbreaks.

Effective surveillance is an essential component of cholera control. Active reporting of suspected cases in areas previously uninfected, with appropriate bacteriological confirmation, will allow the early introduction of the control measures described above. At the international level, systematic reporting of cases to WHO and its collaborative bodies will help to coordinate the international response and limit spread between countries.

Cholera Vaccines

While the formerly used killed whole cell vaccine given parenterally was of only limited efficacy, new oral vaccines have been developed. The principle of these vaccines is to give an oral vaccine providing somatic (O antigen) with and without B subunit toxin immunity in the gut. Cholera vaccines are given orally, have an excellent safety profile and target induction of mucosal immunity. Two oral killed vaccines, prequalified for use by WHO, are licensed and commercially available. Dukoral (whole cell, recombinant B subunit) contains several biotypes and serotypes of *V. cholerae* O1 supplemented with 1 mg per dose of recombinant cholera toxin B subunit. Shanchol (Shantha Biotechnics-Sanofi Pasteur, India) contains several biotypes and serotypes of *V. cholerae* O1 and *V. cholerae* O139 without toxin B subunit. Shanchol is the bivalent vaccine that is internationally available; mORCVAX (VaBiotech, Vietnam) is the locally produced Vietnamese version of this vaccine.[105,109]

The vaccines are administered as 2 or 3 doses depending on age. The vaccines provide 60–85% protective efficacy for 2–3 years, although protection among young children is shorter. There is some evidence of herd protection when vaccine coverage rates are high.[108,109]

Several live attenuated oral cholera vaccines have also been developed, including CVD 103-HgR, Peru-15 and others. These genetically modified vaccine strains do not express cholera toxin. These vaccines have been shown to be safe and immunogenic in volunteer studies, but CVD 103-HgR failed to show protection in a field study in an endemic setting. Peru-15 is safe and immunogenic in different age groups in Bangladesh, but has not yet been tested in field studies.[105]

NON-CHOLERA VIBRIOS

Vibrio spp. other than *V. cholerae* O1 and O139 may cause diarrhoeal diseases in the tropics but are rarely associated with extensive outbreaks. Five species have been associated with diarrhoeal diseases: *V. cholerae* non-O1, *V. parahaemolyticus*, *V. fluvialis*, *V. hollisae* and *V. mimicus*. Among *V. cholerae* O1 strains, some have been isolated that are non-toxigenic but cause diarrhoea. They have been isolated from 1–3% of patients admitted to the cholera hospital in Dhaka, Bangladesh. *V. parahaemolyticus* is principally associated with seafoods. *V. fluvialis* has been implicated in an outbreak of diarrhoeal disease in Bangladesh. Few data are currently available on the prevalence of these vibrios in most tropical countries.

Bacteroides fragilis

Bacteroides fragilis is the only strain of *Bacteroides* spp. associated with diarrhoeal disease. Toxin-producing strains of *B. fragilis*, termed enterotoxigenic *Bacteroides fragilis* (ETBF), are an established cause of diarrhoeal disease in humans. The clinical syndrome associated with ETBF diarrhoeal disease consists of abdominal pain, tenesmus and inflammatory diarrhoea.

ETBF strains have a conjugative transposon containing a pathogenicity island with a distinct virulence gene encoding a 20-kDa metalloprotease toxin called the *B. fragilis* toxin or BFT (also known as fragilysin). BFT is secreted and is detectable in stool. Three subtypes of BFT (BFT-1, BFT-2 and BFT-3) have been identified, with BFT-1 expressed by about two-thirds of ETBF isolated around the globe followed by BFT-2 (25%) and BFT-3 (~10% and mostly identified in South-east Asia). BFT stimulates the cleavage of intercellular adhesion protein E-cadherin on colonic epithelial cells, resulting in increased human colon permeability and activates nuclear factor-kappa B signalling, resulting in proinflammatory cytokine secretion by colonic epithelial cells.[110]

In an observational study of children and adults with acute diarrhoeal illnesses in Dhaka, Bangladesh,[111] ETBF was identified to cause a clinical syndrome with marked abdominal pain and nonfebrile inflammatory diarrhoea in both children (age >1 year) and adults. Faecal leukocytes, lactoferrin and proinflammatory cytokines [interleukin (IL)-8, tumour necrosis factor-α] as well as systemic and faecal anti-BFT responses (IgA, IgG) increased rapidly in ETBF-infected patients. ETBF have also been identified as a cause of travellers' diarrhoea.

Laribacter hongkongensis

This recently described bacterium is a cause of gastroenteritis and traveller's diarrhoea.[112] Although first described in Hong Kong, cases of infection have occurred in mainland China, Japan, Switzerland, Cuba and Tunisia. This bacterium is found in freshwater fish and infection is associated with consumption of improperly cooked fish or poor hygiene in kitchens where raw freshwater fish are handled. It can cause either a watery (80% of cases) or bloody (20%) diarrhoea. Treatment is by rehydration. It is susceptible to fluoroquinolones, co-amoxiclav and aminoglycosides, but resistant to all cephalosporins, mediated by a β-lactamase.

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