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39 INFECTIOUS DIARRHEA

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In the United States, an estimated 21 to 37 million episodes of diarrhea occur annually in children younger than 5 years of age.[1] Ten percent of these children are seen by a physician, more than 200,000 are hospitalized, and between 300 and 400 die from the illness. Worldwide, the number of childhood deaths from diarrhea is higher than 4 million per year.

Knowledge of diarrheal disease has increased remarkably during the past few decades.[2] This increased understanding of pathogenic mechanisms has led to improvements in therapy. This chapter discusses the major viral and bacterial agents of infectious diarrhea, including their epidemiology, pathogenesis, clinical manifestations, diagnosis, and therapy.

VIRAL GASTROENTERITIS

Diarrheal disease caused by viral agents occurs far more frequently than does similar disease of bacterial origin. In fact, viral gastroenteritis is the second most common illness in the United States, after the common cold.[3] Despite the frequent occurrence of viral enteritides, the identification of a specific virus as causative agent is a relatively recent development. Rotavirus and a number of other small round structured viruses have been identified as a major cause of nonbacterial gastroenteritis in children and adults. This discussion focuses on these established pathogens, then continues with a brief summary of several newer viral enteropathogens and the current status of several candidate pathogens.

Rotavirus

Rotavirus was first identified as a specific viral pathogen in duodenal cells of children with diarrhea by Bishop and associates in 1973. Subsequent studies indicated that rotavirus is responsible not only for more cases of diarrheal disease in infants and children than any other single cause but also for a significant portion of deaths caused by diarrhea in both developed and developing countries throughout the world.[4] Rotavirus is responsible for 20 to 70% of hospitalizations for diarrhea among children worldwide.[5] Compared with other causes of gastroenteritis, rotavirus is more frequently associated with severe symptoms.[6] Before the initiation of the rotavirus vaccination program in 2006, nearly every child in the United States was infected with rotavirus by age 5 years.[7]

Virology

The genus *Rotavirus* is classified as a member of the family Reoviridae of the RNA viruses. Rotaviruses are round particles 68 nm in diameter and are composed of two separate shells (capsids). The capsids surround a 38-nm icosahedral core structure, which in turn encloses the 11 double strands of RNA in the core. This structure gives the virus its characteristic appearance of a wide-rimmed wheel with spokes radiating from the hub, from which its name was derived (*rota* is Latin for "wheel").[8]

Rotaviruses are classified based on antigenic properties of various proteins found in the capsid structure. The VP6 protein on the inner capsid of the virus determines the rotavirus group.[9] Most viruses infecting humans are classified as group A, although rotaviruses from groups B and C have occasionally been associated with human diarrheal disease as well. The next level of classification is the subgroup, which is determined by other antigenic differences among the VP6 proteins. At least two subgroups are known to exist.[9] Subgroup typing has proved important in the study of patients who experience more than one episode of rotaviral infection. In these patients, recurrent infections usually but not necessarily involve agents of different subgroups, which suggests that subgroup antigens are not sufficient for inducing the production of protective antibodies.[10] Finally, the rotaviruses are classified into a variety of serotypes based on the antigenic differences of VP7 glycoprotein or the VP4 protease-sensitive hemagglutinin proteins that are found in the outer capsid.[11] VP4 is designated as the P antigenic protein because it is cleaved by the protease trypsin at the intestinal level, and VP7 is designated as the G antigenic protein because it is a glycosylated structure. There are at least 42 different G/P strains with different serotype combinations. However, five serotypes, G1P8, G2P4, GP8, G4P8, and G9P8, are the predominant circulation rotavirus G/P serotypes.[12] The prevalence of serotypes can fluctuate from year to year,[13] and although the five most common serotypes are responsible for approximately 95% of infections worldwide, there are substantial geographical differences. For example, in a recent global study, G1P8 was responsible for more than 70% of infections in North America, Australia, and Europe but less than 30% in South America, Asia, and Africa.[14]

Epidemiology

Rotavirus infection appears to occur throughout the world. In temperate climates, a sharp increase in incidence of cases occurs during the winter months.[4] In the United States, the peak rotavirus season begins in November in the Southwest and ends in the Northeast in April.[4] In the tropics, year-round transmission occurs, with seasonal variation in some areas.[15] Transmission is primarily from person to person, through contact with feces or contaminated fomites. Respiratory transmission has been suggested but not proved.[16] Rotavirus is highly contagious because very few infectious virions are needed to cause disease in susceptible hosts.[17]

Although the virus may affect all age groups, it most commonly produces disease in children between 6 and 24 months of age. Before vaccination, most children developed rotavirus antibodies by the age of 2 years, which helps to explain the observed decreased incidence of rotaviral infection in later

childhood. Rotavirus infection also occurs in adult populations with approximately half the frequency seen in children. Those adults whose children had rotavirus were more likely to be infected than were adults without infected children.[18] Most adults found to have rotavirus infection were asymptomatic; if symptoms were present, they were generally mild. This would seem to indicate that the antibody acquired earlier in life provides protective benefit.

The other age group that appears to have relative protection from rotavirus infection is the neonate. The virus can be found in stool samples from asymptomatic neonates. Neonatal epidemics of rotavirus excretion have been described in which approximately half of the nursery patients examined were found to have rotavirus. Many of these infants were asymptomatic, and those with disease had only mild symptoms.[19,20] Breast-fed infants are less likely to be infected, and, when infected, these infants are apparently less likely than their bottle-fed counterparts to suffer symptoms of disease. This may reflect the protective effect of maternal antibodies in colostrum and breast milk.[21] Nosocomial spread of rotaviral illness among hospitalized infants has also been documented.[22]

Factors associated with increased risk for hospitalization for rotavirus gastroenteritis among U.S. children include lack of breast-feeding, low birth weight, day-care attendance, the presence of another child younger than 24 months in the household, and having Medicaid or no medical insurance.[23]

Clinical Manifestations

Once a susceptible patient has come in contact with rotavirus, a 48- to 72-hour incubation period occurs before the onset of symptoms.[16] Illness typically begins with the sudden onset of diarrhea and vomiting, and fever is present in most patients.[16] The diarrhea is usually watery and rarely may be associated with gross or occult blood in the stool.[24] The fluid loss from diarrhea and vomiting may be severe enough to cause dehydration. Diarrhea caused by rotavirus usually lasts from 2 to 8 days.[25] Shedding of virus into the intestinal lumen begins about 3 days after infection and may persist for as long as 3 weeks.[26] A comparison of the characteristics of rotaviral infections with those of other enteric viruses is presented in Table 39-1. In addition to gastrointestinal symptoms, patients with rotavirus often have respiratory tract symptoms.[16] Unlike fever and vomiting, none of the respiratory manifestations associated with rotavirus infection are helpful in the recognition of rotaviral disease.[27] The clinical symptoms of rotavirus infection are more severe in patients with underlying malnutrition. In the malnourished murine rotavirus model, a smaller inoculum is required for infection, less time is required for incubation, and the symptoms are more severe.[28] In addition, rotavirus replication can occur in the liver and kidney, at least in immunocompromised hosts.[29] Children and adults who are immunocompromised because of congenital immunodeficiency or because of bone marrow or solid organ transplantation sometimes experience severe or prolonged rotavirus gastroenteritis.[7] The severity of rotavirus disease among children infected with human immunodeficiency virus (HIV) is thought to be similar to that among children without HIV infection.[7]

Pathophysiology

Rotavirus invades the villus intestinal epithelial cells and replicates, causing cell death and sloughing. Histologically, this is manifest as blunting of the intestinal villi, and in response to the loss of villus cells, there is crypt hypertrophy. The lytic infection of highly differentiated absorptive enterocytes and the sparing of undifferentiated crypt cells results in both a loss of absorptive capacity with "unopposed" crypt cell secretion (causing secretory diarrhea) and loss of brush border hydrolase activity (causing osmotic diarrhea).

Another possible mechanism for rotaviral diarrhea also has been demonstrated. The rotavirus nonstructural glycoprotein NSP4 has been shown to mediate age-dependent intestinal secretion in mice.[30] The relevance of this novel viral enterotoxin to human rotaviral infection is uncertain. Other models, including vasoactive inflammatory agents, have also been proposed; consistent with this, in rotavirus infection there may be an increase in the number of inflammatory cells in the lamina propria. Disease effects are apparently limited to the duodenum and the proximal jejunum,[16] because studies in patients with known rotavirus disease have yielded normal gastric and rectal biopsies.[31]

Diagnosis

Rotavirus was initially linked to acute gastroenteritis through electron-microscopic evidence of viral particles in biopsy specimens of affected patients. This technique continues to be used in rotavirus detection, especially in conjunction with monoclonal or polyclonal antibodies (immunoelectron microscopy).[25] The obvious drawback of this approach is the need for specialized personnel and equipment. Consequently, a variety of immunoassays have been developed for detecting group A rotavirus antigen in stool[31]; most immunoassays have sensitivities and specificities in the range of 90%.

Treatment

Currently, supportive care with oral or intravenous rehydration is the mainstay of therapy.[32] Although novel antisecretory therapies have been reported,[33] no antiviral agents effective against rotavirus have yet been developed. However, probiotic therapy has been shown to be effective in preventing and treating rotaviral infection. Treatment with *Lactobacillus* GG has been shown to shorten the course of rotaviral diarrhea by at least 1 day.[34-36] In addition, other probiotic agents (*Bifidobacterium bifidum* and *Streptococcus thermophilus*) have been shown to prevent diarrheal disease and shedding of rotavirus in a chronic hospital setting when given to formula-fed infants.[36] Oral administration of immunoglobulin has been shown to promote faster recovery

TABLE 39-1. **Viral Enteric Pathogens**

Virus	Predominant Age Group Affected	Seasonality	Duration of Symptoms
Rotavirus	6-24 months	↑ in winter months	2-8 days
Norovirus/caliciviruses	Older children, adults, infants	Winter and summer	12-48 hours
Enteric adenovirus	<2 years	↑ in summer months	Up to 14 days
Astrovirus	1-3 years	Unknown	1-4 days

from rotaviral infection[37]; this therapy should be reserved for severely affected hospitalized infants.

Prevention

In infants, natural rotavirus infection confers protection against subsequent infection. This protection increases with each new infection and reduces the severity of diarrhea.[38] A rotavirus vaccine (Rotashield J, Wyeth-Ayerst, St. David's, PA) was approved for use in the United States and was placed on the American Academy of Pediatrics' recommended vaccination schedule. Although the vaccine was efficacious, an increased incidence of intussusception within 2 weeks of receiving the vaccine was identified by the Vaccine Adverse Event Reporting System (VAERS), leading to voluntary withdrawal by the manufacturer.[39]

Two different rotavirus vaccine products are licensed and widely used in infants in the United States; they differ in composition and schedule administration. Safety and efficacy has been demonstrated for both vaccines; there is 85 to 98% protection against severe rotavirus disease and 74 to 87% protection against rotavirus disease of any severity through at least the first rotavirus season.[7] Neither vaccine was associated with intussusception, and the Advisory Committee on Immunization Practices (ACIP) does not express a preference for either one.[7]

Pentavalent Human-Bovine Reassortant Rotavirus Vaccine (RotaTeq [RV5]). Licensed in the United States in 2006, RotaTeq is a live, oral vaccine that contains five reassortant rotaviruses developed from human and bovine parent rotavirus strains (G1,G2,G3,G4, and P1A). The efficacy has been evaluated in two phase III trials among healthy infants.[40,41] The vaccine is to be administered orally in a three-dose series at ages 2, 4, and 6 months with a minimum age for first dose at 6 weeks and maximum at 14 weeks and 6 days. The minimal interval between doses is 4 weeks and maximum age for last dose 8 months.[7]

Monovalent Human Rotavirus Vaccine (Rotarix [RV1]). Licensed in the United States in 2008, Rotarix is a live, oral vaccine that contains a human rotavirus strain (G1P1A). The efficacy has been evaluated in two phase III trials.[42,43] The vaccine is to be administered orally in a two-dose series at ages 2 and 4 months with the same minimum and maximum age ranges and intervals as RotaTeq.[7]

Early success from the vaccines has been documented; the National Respiratory and Enteric Virus Surveillance System (NREVSS) and the New Vaccine Surveillance Network (NVSN) indicated that the onset and peak of the 2008 rotavirus season were delayed by 15 and 8 weeks, respectively. as compared with the six previous consecutive seasons.[44] Further data indicate that the number of tests positive for rotavirus during the 2008 season decreased by more than two thirds as compared with the seven preceding rotavirus seasons.[7]

Small Round Structured Viruses

Caliciviruses

"Winter vomiting disease" was thought to be caused by nonbacterial gastroenteritis for decades before an etiologic agent was identified from an outbreak, in 1968, in Norwalk, Ohio. In this outbreak, only some of the patients had diarrhea; the predominant clinical manifestation was vomiting and nausea. Virus particles were visualized by immune electron microscopy on fecal material derived from the Norwalk outbreak. This represented the first definitive association between a specific virus (Norwalk virus) and acute gastroenteritis. Subsequently a number of similar etiologic agents were identified; before the cloning of the prototype Norwalk virus genome,[45] these viruses, which were a group of morphologically diverse, positive-stranded RNA viruses that caused acute gastroenteritis, were identified as Norwalk-like agents. These organisms were also named for the communities in which they were first isolated (e.g., Montgomery County, Hawaii, Snow Mountain, Taunton, Otofuke, and Sapporo viruses). Based on reverse transcription–polymerase chain reaction (RT-PCR), the sequence structure of these viruses has enabled their classification as human caliciviruses (HuCV). Human caliciviruses are now recognized as a leading cause of diarrhea worldwide among persons of all ages.[46]

With the use of molecular tools, HuCV have now been preliminarily classified into four genotypes, represented by Norwalk virus, Snow Mountain agent, Sapporo virus, and hepatitis E virus.[47,48] Recently the nomenclature of two genotypes has changed, renaming Norwalk virus as norovirus and Sapporo virus as sapovirus.[49] This HuCV classification system may allow the development of assays based on recombinant HuCV antigens or PCR products rather than the current cumbersome classification schemes that rely on human reagents (convalescent outbreak sera) of varying sensitivity and specificity. Molecular tools have already allowed the identification of HuCV as agents of both pediatric and adult viral gastroenteritis in foodborne outbreaks as well as outbreaks in nursing homes, hospitals, and a university setting. Despite the potential for future understanding of the contribution of individual HuCV to outbreaks of nonbacterial gastroenteritis, Norwalk virus still remains the prototypic agent of HuCV, and it is described in greater detail in the following section.

Norovirus

Epidemiology. Norovirus is worldwide in distribution. Of patients exposed to norovirus either naturally or experimentally, 50% develop clinical symptoms.[50] Studies evaluating the prevalence of anti-norovirus antibody among populations of various age groups initially demonstrated that the group from 3 months to 12 years of age had only a 5% antibody-positive rate. More recent epidemiologic studies, using baculovirus-expressed recombinant norovirus antigen in an enzyme-linked immunosorbent assay (ELISA), have demonstrated a serologic response in 49% of Finnish infants between 3 and 24 months of age.[51] These data contradict previous beliefs that norovirus most often caused disease in older children and adults.

Transmission of norovirus is most often fecal-oral. Unlike rotavirus, this usually involves the spread of infection to a large population through a common source rather than from direct, person-to-person contact. In one outbreak, an infected bakery employee transmitted the virus through food products to approximately 3000 people.[52] Outbreaks have also been related to ingestion of raw oysters and clams and to contaminated water supplies. Spread of this disease has been documented in closed-in populations such as those in long-term care facilities and cruise ships.[53] In addition to its fecal-oral spread, there is some evidence that norovirus is transmitted through a respiratory route in the form of aerosolized particles from vomitus. Contamination of environmental surfaces with norovirus has been documented during outbreaks.[54] Although previously

referred to as "winter vomiting disease," norovirus produces outbreaks of disease that can occur throughout the year.[55] Several characteristics of norovirus facilitate their spread in epidemics: (1) low infectious dose (fewer than 10 viral particles), (2) prolonged viral shedding, (3) stability of the virus in relatively high concentrations of chlorine and a wide range of temperatures, and (4) the fact that repeated infections can occur with reexposure.[46]

Pathophysiology. The histologic changes induced by norovirus in an infected host have been studied in small bowel biopsies from infected volunteers. Those volunteers who remained free of clinical symptoms had normal biopsy specimens, whereas those with symptoms exhibited marked, but not specific, changes, including focal areas of villous flattening and disorganization of epithelial cells. On electron microscopy, microvilli were shortened, and there was dilatation of the endoplasmic reticulum. These volunteers had repeat biopsies 2 weeks after the illness, and normal histology was again present. Other investigators have demonstrated the presence of normal gastric and rectal histology in patients affected by norovirus as is typical of viral gastroenteritis. Using norovirus virus-like particles (derived from capsid proteins) researchers have recently demonstrated that human histo-blood group antigens may act as receptors for norovirus infection[56,57] and may explain the varying host susceptibility observed in outbreaks and volunteer studies.[58]

Clinical Manifestations. The clinical manifestations of disease produced by the norovirus include nausea, vomiting, and cramping abdominal pain (see Table 39-1). Diarrhea is said to be a less consistent feature of this illness. In the original outbreak, only 44% of patients experienced diarrhea, whereas 84% had vomiting. Other studies, however, have found that diarrhea occurs in most children and experimentally infected adult volunteers who become ill from this virus. Fever occurs in approximately one third of affected patients, but respiratory symptoms are not typically a part of this illness. An incubation period of approximately 24 to 48 hours has been noted before the onset of symptoms,[50] and symptoms persist for 12 to 48 hours. The typical symptoms of infection are in part also seen in premature infants but with a huge variety of clinical courses including abdominal distention, apnea, and sepsis-like appearance.[59]

Diagnosis and Treatment. Norwalk virus could be detected in fecal samples for a median of 4 weeks and for up to 8 weeks after virus inoculation; peak virus titers are most commonly found in fecal samples collected after resolution of symptoms, and presymptomatic shedding was more common in persons who did not meet the definition of clinical gastroenteritis.[60] RT-PCR assays have been developed for detection of noroviruses in clinical and environmental specimens, such as water and food.[61,62] RT-PCR followed by nucleotide sequencing has been useful in epidemiologic studies, and also various commercial stool enzyme immunoassay (EIA) detection methods have been developed[46]; the sensitivity is genotype dependent.[63] A rapid and accurate diagnostic assay is not widely available, but the presence of four epidemiologic features of norovirus disease can be useful in confirming norovirus as a cause of outbreaks: (1) vomiting in more than half of affected persons, (2) mean incubation period of 24 to 48 hours, (3) mean duration of illness of 12 to 60 hours, and (4) absence of bacterial pathogen in stool culture.[64]

The treatment for norovirus is supportive; oral rehydration solutions are used if necessary. Significant dehydration is uncommon, and the need for hospitalization is rare. A number of candidate vaccines are currently being evaluated.

Enteric Adenovirus

The enteric adenoviruses are among the more recently recognized viral pathogens that cause acute gastroenteritis. Adenoviruses are a large group of viruses long recognized for their role in the pathogenesis of respiratory infections and keratoconjunctivitis. Most of the 47 serotypes are known to be shed in the feces of infected patients. In patients with predominantly gastrointestinal symptoms, the organisms are detectable by electron microscopy of stool samples; however, they fail to grow in standard tissue culture conditions. Their unique cell culture requirements allow for the differentiation of nonenteric adenoviruses from the enteric serotypes (Ad40 and Ad41), which are recognized to be among the common causes of viral childhood gastroenteritis.[65]

Infection with enteric adenoviruses apparently occurs throughout the year, with only slight seasonal variation.[66] This disease tends to affect predominantly younger children, with most patients being younger than 2 years of age.[66,67] Enteric adenovirus is spread by the fecal-oral route. Transmission of the disease to family contacts is unusual.

Diarrhea is the most commonly reported symptom of enteric adenoviral infection. In contrast with diarrhea from other viral enteritides, diarrhea from enteric adenovirus typically persists for a prolonged period, sometimes as long as 14 days. Viruses may be excreted in the feces of infected patients for 1 to 2 weeks. Vomiting frequently occurs but is usually mild and of a much shorter duration than is the diarrhea. Dehydration has been seen in approximately half of affected patients, and hospitalization is sometimes necessary. The frequency of association of respiratory symptoms with enteric adenovirus infection is unclear.[67]

The diagnosis of enteric adenovirus is best made by electron microscopy or immunoelectron microscopy of stool samples or from intestinal biopsy specimens. ELISA and PCR techniques have also been used successfully in enteric adenovirus diagnosis. Treatment is mainly supportive, and oral rehydration solutions are useful in cases of dehydration.

Astrovirus

Astrovirus, similar to HuCV, is a single-stranded RNA virus grouped with the small round structured viruses. However, the recently derived sequence of the astrovirus RNA genome reveals that this agent is sufficiently different to be classified in its own family as Astroviridae.[68] Astrovirus is worldwide in distribution and tends to infect mainly children in the 1- to 3-year age group. In controlled studies in Thailand, astrovirus infection was the second most common cause of enteritis, after rotavirus infection, in symptomatic children.[69] Astrovirus infection occurred in 9% of children with diarrhea, compared with 2% of controls. Comparable findings have been reported in day-care centers in North America and Japan. Most children infected with astrovirus develop symptoms. Vomiting, diarrhea, abdominal pain, and fever all are commonly seen with infection by this agent, and symptoms typically last 1 to 4 days. Spread of the virus may occur via the fecal-oral route from person-to-person contact or

through contaminated food or water. Asymptomatic shedding of astrovirus has also been reported.

Other Viruses

A variety of other viruses are being studied to determine what role, if any, they may play in the pathogenesis of human enteric infections. With the exception of those viruses previously discussed in detail, insufficient data are available to ascertain clinical and epidemiologic differences, if any, among the various small round viruses.

Pestivirus, a single-stranded RNA virus of the togavirus family, has been found in the feces of 24% of children living on an American Indian reservation who had diarrhea attributable to no other infectious agent.[70] These children experienced only mild diarrhea but had more severe respiratory complaints.

Coronavirus is known to cause an upper respiratory illness in humans and has been shown to cause diarrhea in some animals.[71] The role of this agent in human diarrheal disease is unclear, and at least one study found coronavirus more commonly in children without diarrhea than in those who were ill.[72] Coronavirus was implicated in an outbreak of necrotizing enterocolitis.[73]

Toroviruses are pleomorphic viruses recognized to cause enteric illness in a variety of animals. Members of this group, originally described in Berne, Switzerland, and Breda, Iowa, and named for those cities, have been seen in the feces of humans with diarrheal disease.[74] Because of the pleomorphic structure of toroviruses, electron microscopy was inadequate to prove an etiopathogenic role of these viruses in diarrheal disease. The more recent findings of torovirus-like particles by immunoassay, using validated anti-Breda virus antiserum, lends additional weight to the hypothesis that these are agents of human gastroenteritis.[75] Their causative role in human disease, however, remains unproved. Similarly, picobirnavirus is known to cause disease in animals and has been isolated from stools of humans with diarrheal illness.[76]

Cytomegalovirus has been associated with enteritis and colitis. Except for Ménétrier's disease, caused by gastric cytomegalovirus infection, enteritis and colitis seem to occur almost exclusively among immunocompromised patients. In this population, cytomegalovirus causes viremia and is carried by the blood stream to a variety of sites, including organs of the gastrointestinal tract. Diagnosis may be made by virus detection in feces, by demonstration of typical cytomegalic inclusion cells, or by in situ hybridization.[66]

BACTERIAL GASTROENTERITIS

Host-Defense Factors

For an infecting bacterial agent to cause diarrhea, it must first overcome the following gastrointestinal tract defenses: (1) gastric acidity, (2) intestinal motility, (3) mucus secretion, (4) normal intestinal microflora, and (5) specific mucosal and systemic immune mechanisms. Gastric acidity is the first barrier encountered by infecting organisms. Many studies have demonstrated the bactericidal properties of gastric juice at pH less than 4. In patients with achlorhydria or decreased gastric acid secretion, the gastric pH is higher, and this bactericidal effect is diminished. Gastric acidity serves to decrease the number of viable bacteria that proceed to the small intestine.

Organisms surviving the gastric acidity barrier are trapped within the mucous layer of the small intestine, facilitating their movement through the intestine by peristalsis. If motility in the intestine is abnormal or absent, organisms are more readily able to initiate the infectious process. Some organisms can elaborate toxic substances that impair intestinal motility. Increased intestinal peristalsis, which occurs during some enteric infections, may be an attempt by the host to rid itself of infective organisms.

In addition to its role in conjunction with intestinal motility, mucus also serves to provide a nonspecific barrier to bacterial proliferation and mucosal colonization. This barrier has been shown to be effective in preventing toxins from exerting their effects. Exfoliated mucosal cells trapped in the mucous layer may trap invading microorganisms. Mucus also contains carbohydrate analogues of surface receptors, which may prevent invading organisms from binding to actual receptors.

The normal endogenous microflora of the gut serves as its next line of defense. Anaerobes, which are a large component of the normal flora, elaborate short-chain fatty acids and lactic acid, which are toxic to many potential pathogens. In breastfed infants, this line of defense is enhanced by the presence of anaerobic lactobacilli, which produce fermentative products that act as toxins to foreign bacteria. Further evidence in support of the importance of endogenous microflora is the increase in susceptibility to infection after one's normal flora has been reduced by antibiotic administration, as is seen with *Clostridium difficile* infection.

The most complex element in the host-defense armamentarium involves the mucosal and systemic immune systems. Both serum and secretory antibodies may exert their protective effects at the intestinal level, even though the serum components are produced outside the gut. An immune response may be *specific* to a particular infective agent or *generalized* to a common group of bacterial antigens.

Mechanisms of Bacterial Disease Production

Bacteria have developed a variety of virulence factors (Table 39-2) to overcome host defense mechanisms: (1) *invasion* of the mucosa, followed by intraepithelial cell multiplication or invasion of the lamina propria; (2) production of *cytotoxins*, which disrupt cell function via direct alteration of the mucosal surface; (3) production of *enterotoxins*, polypeptides that alter cellular salt and water balance yet leave cell morphology undisturbed; and (4) *adherence* to the mucosal surface with resultant flattening of the microvilli and disruption of normal cell functioning. Each of the bacterial virulence mechanisms acts on specific regions of the intestine. Enterotoxins are primarily effective in the small bowel but can affect the colon; the effects of cytotoxins and direct epithelial cell invasion occur predominantly in the colon. Enteroadhesive mechanisms appear to function in both the small intestine and colon.

Salmonella

Members of the species *Salmonella* are currently recognized as the most common cause of bacterial diarrhea among children in the United States. Surveillance data from the Centers for Disease Control and Prevention show that in 2008 the incidence of *Salmonella* was 16.2 per 100,000, and although there was an apparent increase in *Salmonella* infections, this rate has not changed significantly over the past 3 years.[77] Infection caused by *Salmonella* may result in several different clinical syndromes, including

TABLE 39-2. **Bacterial Pathogens Grouped by Pathogenic Mechanism**

Invasive	Cytotoxic	Toxigenic	Adherent
Shigella	*Shigella*	*Shigella*	Enteropathogenic *E. coli*
Salmonella	Enteropathogenic *Escherichia coli*	*Enterotoxigenic E. coli*	Shiga toxin-producing *E. coli*
Yersinia enterocolitica	Shiga toxin-producing *E. coli*	*Yersinia enterocolitica*	Enteroaggregative *E. coli*
Campylobacter jejuni	*Clostridium difficile*	*Aeromonas*	Diffusely adherent *E. coli*
Vibrio parahaemolyticus		*V. cholerae* and non-O1 vibrios	

Modified from Cohen MB. Etiology and mechanisms of acute infectious diarrhea in infants in the United States. J Pediatr 1991; 118:S34-S43,[92] with permission.

(1) acute gastroenteritis; (2) focal, nonintestinal infections; (3) bacteremia; (4) asymptomatic carrier state; and (5) enteric fever (including typhoid fever). Each of these entities may be caused by any of the commonly recognized species of *Salmonella*.

Microbiology

Salmonella is a motile, gram-negative bacillus of the family Enterobacteriaceae. It can be identified on selective media because it does not ferment lactose. Three distinct species of *Salmonella* are recognized: *Salmonella enteritidis, Salmonella choleraesuis,* and *Salmonella typhi*. *S. enteritidis* is further subdivided into approximately 1700 serotypes. Each serotype is referred to by its genus and serotype names (e.g., *Salmonella typhimurium*) rather than the formally correct *S. enteritidis*, serotype *typhimurium*. *S. choleraesuis* and *S. typhi* are known to have only one serotype each. The most common serotypes in infants are Typhimurium, Newport, Javiana, Enteritidis, and Heidelberg.[78]

Epidemiology

Salmonella is estimated to cause 1 to 2 million gastrointestinal infections each year in the United States.[79] At Cincinnati Children's Hospital Medical Center, salmonellae are the most commonly isolated bacterial enteropathogens (Figure 39-1). The highest attack rate for salmonellosis is in infancy, with a lower incidence of symptomatic infection in patients older than 6 years of age.[79] Nontyphoidal *Salmonella* is usually spread via contaminated water supplies or foods, with meat, fresh produce, fowl, eggs, and raw milk frequently implicated.

A wide variety of foods have caused outbreaks of salmonella; a large outbreak involved contaminated alfalfa sprouts that were shipped worldwide.[80] Most of the egg-associated outbreaks have involved products such as mayonnaise, ice cream,[81] and cold desserts, in which salmonella can multiply profusely and which are eaten without cooking after the addition of, or contamination by, raw egg. Although "shell" eggs are frequently contaminated, the number of bacteria in infected eggs is often near or below the human infective dose. In contrast, with a generation time of 80 minutes at 20° C, one bacterium can become a billion in 40 hours, and with a generation time of 40 minutes at 25° C, it can do so in 20 hours.

Although any of these food sources may become contaminated through contact with an infected food handler, the farm animals themselves are often infected. Pets, notably cats, turtles, lizards, snakes, and chicks, may also harbor *Salmonella*. Person-to-person spread of infection also occurs and is especially common in cases involving infants. A population-based case-control study was done in infants less than 1 year of age and identified the following risk factors: (1) travel outside the United States, (2) attending day care with a child with diarrhea, (3) riding in a shopping cart next to meat or poultry; and (4) exposure to reptiles. Breast-feeding was found to be protective. [82]

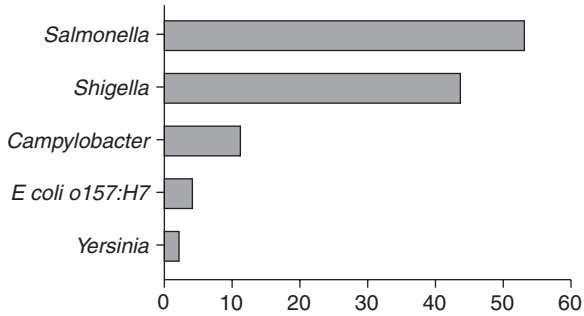

Figure 39-1. Bacterial enteropathogens isolated at Cincinnati Children's Hospital Medical Center (CCHMC) in the year 2008. In addition to stool cultures above, 256 specimens tested positive for *C. difficile* by toxin assay in the year 2008. A total of 4601 stool cultures and 2950 tests for *C. difficile* were sent in 2008. Data from Infection Control Office, CCHMC.

Pathogenesis

Inocula of fewer than 10^3 salmonellae are probably sufficient to cause disease.[83] Patients in whom host defenses are diminished are more likely to develop clinical manifestations of the disease. This has been demonstrated in patients who have reduced levels of gastric acid. Patients with lymphoproliferative diseases and hemolytic diseases, especially sickle cell anemia, are more likely to experience severe disease and develop complications from *Salmonella* infection. The mechanisms for this increased susceptibility may involve altered macrophage function, defective complement activation, or damage to the bones from thromboses.

Having overcome host defenses, *Salmonella* produces disease through a process that begins with colonization of the ileum and the colon. The organisms next invade enterocytes and colonocytes and proliferate within epithelial cells and in the lamina propria (Figure 39-2). From the lamina propria, *Salmonella* may then move to the mesenteric lymph nodes and eventually to the systemic circulation, causing bacteremia. Because these organisms invade enterocytes and colonocytes, both enteritis, with watery diarrhea, and colitis, with bloody diarrhea, may result. This multistage infection of the host is directed by *Salmonella*-mediated delivery of an array of specialized effector proteins into the eukaryotic host cells via two distinct secretion systems. Additional secretion systems appear to be functional and contribute toward virulence but are not currently well characterized.[84]

Clinical Manifestations

After an incubation period of 12 to 72 hours, *Salmonella* usually produces a mild, self-limited illness characterized by fever and watery diarrhea. Blood, mucus, or both are commonly present in the stool. Bacteremia occurs in approximately 6%

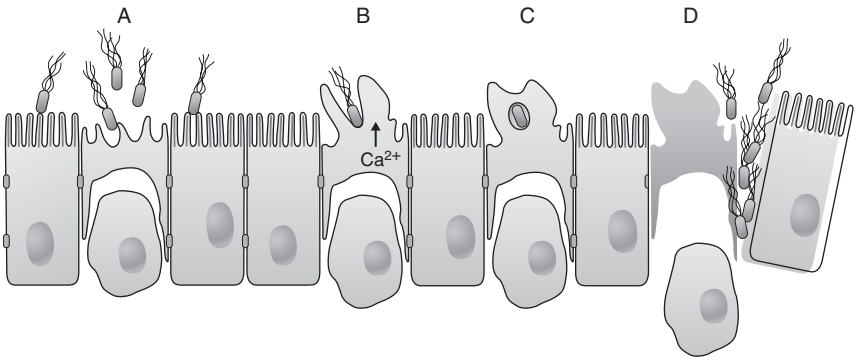

Figure 39-2. Interaction of enteropathogenic *Salmonella* species with the intestinal epithelium. Diagrammed are the interaction and invasion of salmonellae with an M cell and an absorptive epithelial cell overlying the Peyer's patch follicle. *Salmonella* invasion is shown for an M cell. Adherence of salmonellae to an M cell (**A**) is followed by *Salmonella* invasion-induced membrane ruffle (**B**). (**C**), Bacterium localized within an intracellular vacuole. (**D**), Destruction of the invaded M cell followed by an influx of bacteria into the epithelial cell breach and entry into Peyer's patch. From Hromockyj A, Falkow S. Interactions of bacteria with the gut epithelium. In: Blaser MJ, Smith PD, Ravdin JI, et al., eds. Infections of the Gastrointestinal Tract. New York: Raven Press; 1995.[97] Courtesy Brad Jones, PhD.

of *Salmonella* infections in children but much less frequently in adults. Patients may develop nonintestinal sequelae after *Salmonella* infection, including pneumonia, meningitis, and osteomyelitis.

Even in those patients in whom no sequelae occur, excretion of the organisms may persist for several weeks. In patients younger than 5 years of age, the median time of excretion is 7 weeks, with 2.6% of patients continuing to shed organisms for 1 year or longer.[85] Studies have also shown a higher incidence of the carrier state among children with salmonellosis than is seen in adults.[85] Localization of *Salmonella* organisms in chronic carriers is often in the biliary tract and is frequently associated with cholelithiasis.

Diagnosis and Treatment

Diagnosis of *Salmonella* infection can be made through stool or blood culture. Use of enriched media and culture of material from freshly passed stools, rather than from rectal swab, increase the likelihood of recovering the organism.[85] Owing to the increased risk of developing the carrier state, antimicrobial treatment of uncomplicated cases of *Salmonella* gastroenteritis is not recommended. Treatment is recommended in patients at high risk for the development of disseminated disease, including those who are immunocompromised, those with hematologic disease, patients with artificial implants, those with severe colitis, and pregnant women. Treatment is also recommended for patients at any age who appear toxic.

Treatment of all children younger than 1 year of age with salmonellosis remains controversial because of the risk of bacteremia and secondary infections. Antimicrobial therapy is recommended for infants with *Salmonella* bacteremia. Parenteral antibiotics are recommended for any infant (younger than 3 months of age) with a stool culture that is positive for *Salmonella*.[86]

Most *Salmonella* are sensitive to a wide variety of antibiotics, including ampicillin (35 mg/kg [maximum 1 g] per dose, given every 4 hours, intravenously, for 14 days), chloramphenicol (20 mg/kg [maximum 1 g] per dose, given every 6 hours, intravenously or orally, for 14 days), trimethoprim-sulfamethoxazole (trimethoprim, 5 mg/kg [maximum 160 mg], plus sulfamethoxazole, 25 mg/kg [maximum 800 mg] per dose, given every 12 hours, orally, for 14 days), and the third-generation cephalosporins. Resistance to ampicillin is increasing.[87] Ceftriaxone, cefotaxime, or a fluoroquinolone (not approved for use in children younger than 18 years of age) are often effective when resistance to other agents is demonstrated.

A follow-up stool culture usually is not warranted unless the patient is employed in the preparation of food. If evidence of a "cure" is necessary, two to three consecutive negative stool cultures, obtained 1 to 3 days apart, are sufficient.

Typhoid Fever

Although uncommon in the United States, typhoid fever, caused by *S. typhi*, commonly affects children in developing countries. *S. typhi* differs from other salmonellae in that it requires a human host. The disease it causes also differs in severity from the typically mild gastroenteritis caused by other members of the genus; *S. typhi* infection also has a higher case-fatality rate.

Typhoid fever typically begins with a period of fever lasting approximately 1 week. Patients then complain of headache and abdominal pain. Diarrhea is not usually a manifestation of typhoid fever, and many patients experience constipation. Hepatomegaly and splenomegaly have also been frequently noted. The characteristic "rose spots" (palpable, erythematous lesions), typical in adult cases of typhoid fever, occur with far less frequency in pediatric patients. Patients may become chronic carriers.

Diagnosis of typhoid fever is made on the basis of positive blood cultures. *S. typhi* is usually sensitive to several antimicrobial agents, including ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, cefotaxime, and ceftriaxone. Drug choice is based on site of infection and susceptibility of the organism. A recent Cochrane review[88] showed that azithromycin appears to be better than fluoroquinolones in populations with drug-resistant strains and that it may also perform better than ceftriaxone.

Two typhoid vaccines are commercially available; a live, oral Ty21a and injectable Vi polysaccharide. They have been shown to be safe and efficacious and are licensed for people aged more than 2 years.[89,90] Immunization of school-age or preschool-age children is recommended in areas where typhoid fever is shown to be a significant public health problem, particularly where antibiotic-resistant *S. typhi* is prevalent. Vaccination may be offered to travelers to destinations where the risk of typhoid fever is high, especially to those staying in endemic areas for longer than 1 month.[89]

Other vaccines, such as a new modified, conjugated Vi vaccine called Vi-rEPA, are in development and may confer longer immunity.[90]

Shigella

Bacillary dysentery, an illness caused by *Shigella*, was described in ancient Greece. Osler, in 1892, referred to the disease as "one of the four great epidemic diseases of the world." He further

stated: "In the tropics it destroys more lives than cholera, and it has been more fatal to armies than powder and shot." Despite our increased knowledge of the pathogenesis and treatment of shigellosis, this organism continues to be a significant cause of diarrheal disease.

Microbiology

Shigella is a gram-negative, nonmotile, non-lactose-fermenting aerobic bacillus, closely related to members of the genus *Escherichia*. The organisms are classified into four species or groups known as *Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei* (groups A, B, C, and D, respectively). Members of groups A, B, and C exist in numerous serotypes, but only one serotype of group D is known. *S. sonnei* is the most commonly recovered *Shigella* species in the developed world, accounting for 70% of isolates in the United States. *S. dysenteriae* and *S. flexneri* are the most commonly recovered species of *Shigella* in the developing world.[91]

Epidemiology

Shigella is worldwide in its distribution, and the incidence and severity of shigellosis span an equally broad range. In 2008, FoodNet calculated the incidence of *Shigella* infection in the United States to be 6.59 per 100,000.[77] Although *Shigella* occurs much less frequently in the developed world, in some studies it is the second most common pathogen identified in cases of bacterial diarrhea in children aged 6 months to 10 years.[92] It may also be the most common bacterial cause of outbreaks of diarrhea in day-care settings. Outbreaks of shigellosis have also been described in residential institutions and on cruise ships. This disease is endemic on American Indian reservations in the Southwest.

Shigella is predominantly spread via the fecal-oral route, with person-to-person contact the most likely method. Secondary spread to household contacts may occur. The infection may be spread through contamination of food and water, as often occurs in areas of poor sanitation and inadequate personal hygiene.

Risk exposures for cases include international travel in the week before symptom onset, attending or working in day care, contact with a child or household member with diarrheal illness, using untreated drinking water or recreational water, and sexual contact with someone with diarrhea.[78] It is important to know that shigellosis should still be considered in patients with watery diarrhea even without a contact history.[93]

Clinical Manifestations

Patients infected with *Shigella* may experience a mild, self-limited, watery diarrhea that is clinically indistinguishable from gastroenteritis caused by a variety of other agents. The more classic form of shigellosis, however, is bacillary dysentery. This illness usually begins with fever and malaise, followed by watery diarrhea and cramping abdominal pain. By the second day of illness, blood and mucus are usually present in the stools, and tenesmus has become a prominent symptom. At this point, in approximately 50% of affected patients, the stool volume decreases, with only scant amounts of blood and mucus being passed.[91] This pattern of bloody, mucus-containing stools is referred to as *dysentery*. Bacteremia is an uncommon feature of this illness, but several other complications have been reported, including seizures (in children), arthritis, purulent keratitis, and the hemolytic-uremic syndrome (HUS). Nonsuppurative arthritis is the most commonly occurring extraintestinal complication of shigellosis. Patients who carry the histocompatibility locus antigen HLA-B27 may be predisposed to the development of this complication as well as to the development of Reiter's syndrome. The association of seizures with shigellosis was earlier attributed to the neurotoxic effect of the *Shigella* toxin (Shiga toxin). It now seems likely, however, that the seizures may simply represent a subgroup of common febrile seizures and have no direct relation to the effects of Shiga toxin.

Pathogenesis

Shigella has been found to cause disease only in humans and in the higher apes.[91] The organisms are potent, with as few as 10 organisms being able to cause disease in a healthy adult.[91] Patients infected with *Shigella* may excrete 10^5 to 10^8 organisms per gram of feces. This high rate of excretion and the relatively low number of organisms required to produce disease make possible the widespread distribution of disease.

For *Shigella* to exert its pathologic effect on a host, the bacteria must first come into contact with the surface of an intestinal epithelial cell and induce cytoskeletal rearrangements resulting in phagocytosis.[94,95] The bacteria then secrete enzymes that degrade the phagosomal membrane, releasing the bacteria into the host cytoplasm. Intracytoplasmic bacteria move rapidly, in association with a comet tail made up of host-cell actin filaments. When moving bacteria reach the cell margin, they push out long protrusions with the bacteria at the tips that are then taken up by neighboring cells, allowing the infection to spread from cell to cell (Figure 39-3).

Shiga toxin is elaborated by all species, although in greater amounts by *S. dysenteriae* than by other species,[91] and may play a role in the pathogenesis of *Shigella* infection. The toxin has neurotoxic, enterotoxic, and cytotoxic effects.[91] Structurally, it is composed of an active, or A, subunit (molecular weight 32 kDa) surrounded by five binding, or B, subunits (77 kDa).[91] The B subunits bind to cell-specific receptors and are taken up by endocytosis. Within the cells, the B subunits are cleaved away, and the remaining A subunit is shortened by proteolysis. This molecule is thought then to bind to the 60S ribosome and inhibit protein synthesis, leading to cell death and sloughing.[96] This is the presumed mechanism for the cytotoxic effect. An enterotoxic effect of Shiga toxin in the ileum may account for the early watery diarrhea.

Diagnosis and Treatment

In patients with signs and symptoms of colitis, the diagnosis of shigellosis should be considered. Stool culture provides the only definitive means to differentiate this organism from other invasive pathogens. *Shigella* may be cultured from stool specimens or rectal swabs, especially if mucus is present, but there may be a delay of several days from the onset of symptoms to the recovery of organisms. Sigmoidoscopy or colonoscopy typically reveals a friable mucosa, possibly with discrete ulcers. Rectal biopsy may be useful to differentiate shigellosis from ulcerative colitis.

In addition to rehydration, antimicrobial therapy has been recommended for *Shigella* (1) to shorten the course of the disease, (2) to decrease the period of excretion of the organisms, and (3) to decrease the secondary attack rate, because humans provide the only reservoir for the organism. However,

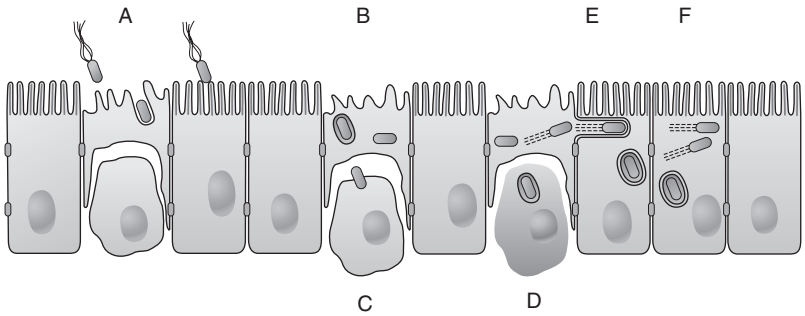

Figure 39-3. Interaction of *Shigella* species with the gut epithelium. Diagrammed is the putative interaction of shigellae with M cells overlying Peyer's patch follicles as well as absorptive epithelial cells. Invasion is diagrammed for an M cell. (**A, B**) Adherence to and intimate association of shigellae with an M cell followed by localization of the invading organism with an intracellular cytoplasmic vacuole. (**C-E**) Bacteria, having transcytosed the M cell, may interact with Peyer's patch macrophages and induce macrophage apoptosis. Bacteria free within the target cell cytoplasm also move within the host cell via an actin-associated tail. (**F**) *Shigella* intercellular invasion through a host cell membrane protrusion, followed by residence of the invading organism within a double-membraned intracellular cytoplasmic vacuole and escape from that vacuole. From Hromockyj A, Falkow S. Interactions of bacteria with the gut epithelium. In: Blaser MJ, Smith PD, Ravdin JI, et al., eds. Infections of the Gastrointestinal Tract. New York: Raven Press; 1995,[97] with permission.

handwashing, rather than use of antimicrobials, is the most effective method to prevent person-to-person spread. Those clinicians who advise against the routine treatment of shigellosis with antibiotics argue that (1) the disease is most often self-limited and (2) the use of antibiotics may facilitate the development of resistant strains and may increase the likelihood of developing HUS.

We recommend antibiotic therapy only for patients who are severely ill at the time of diagnosis or who remain ill at the time of identification of *Shigella* in a stool culture. A wide range of antibiotics has been used to treat *Shigella,* necessitated by the development of resistant strains. Currently, the agent of choice is trimethoprim-sulfamethoxazole (trimethoprim, 5 mg/kg [maximum 160 mg], plus sulfamethoxazole, 25 mg/kg [maximum 800 mg] per dose, given every 12 hours, orally or intravenously, for 5 days). Ampicillin (25 mg/kg [maximum 500 mg] per dose, given every 6 hours, orally or intravenously, for 5 days) may be used if local strains are typically susceptible.[97] Amoxicillin is ineffective against *Shigella*. Nalidixic acid (55 mg/kg per day given every 6 hours for 5 days) has proved effective. Cefixime and ceftriaxone are alternative agents for resistant organisms.[91] Tetracycline, ciprofloxacin, and norfloxacin have been used successfully for the treatment of *Shigella*, but these agents are approved for use only in adult patients. Multidrug-resistant strains have occurred in Latin America, central Africa, and Southeast Asia.[98]

Development of a vaccine for shigellosis continues to be a challenge. These efforts include vaccines using a modified *Escherichia coli* strain; one using a mutant strain of *S. flexneri*, which lacks the ability to proliferate intracellularly; and one based on a strain with mutations in its virulence genes. Vaccine development continues to be limited by the lack of a suitable animal model.[99]

Campylobacter

Campylobacter is a gram-negative, motile, curved or spiral-shaped rod, exhibiting a "seagull" appearance when identified in stained stool smears. Multiple species of *Campylobacter* have been recognized, including *Campylobacter jejuni, Campylobacter fetus, Campylobacter coli,* and *Campylobacter laridis,* with *C. jejuni* being the one most commonly associated with disease in humans. *Campylobacter upsaliensis* has been reported as another member of this group that causes diarrhea,[100] and it seems probable that still others may be identified.

Epidemiology

Campylobacter is recognized to be worldwide in distribution. In developing countries, *Campylobacter* is a significant bacterial cause of diarrhea in children younger than 2 years of age, yet it rarely occurs in developing nations in older children and adults. When infection does occur in the population older than 2 years of age, it tends to be asymptomatic.[101] It is likely that patients in these countries are infected with *Campylobacter* early in life and then develop immunity, thus making asymptomatic infection more typical in older children and adults.

In the industrialized world, most patients infected with *Campylobacter* develop symptoms.[101] The number of *Campylobacter* infections in these countries is now recognized to be quite high, with some studies finding this organism to be the most common cause of bacterial diarrhea. *Campylobacter* tends to infect people in two distinct age groups: children in the first year of life and young adults. *Campylobacter* spp. is the most common cause of bacterial enteric infections in the United States, causing an estimated 2 million infections annually.[102]

Campylobacter may be spread by direct contact or through contaminated sources of food and water. Milk, meat, and eggs, especially if undercooked, have been implicated in outbreaks. These sources may be contaminated from human fecal shedding, or the organisms may be harbored in the asymptomatic farm animals. *Campylobacter* is commonly spread among populations of children in day-care centers. A population-based case-control study showed that risk factors for campylobacteriosis were drinking well water, eating fruits and vegetables prepared in the home, having a pet in the home with diarrhea, visiting or living on a farm, riding in a shopping cart next to meat or poultry, and traveling outside of the United States. Infants with campylobacteriosis were less likely to be breast-fed or to be in a household where hamburger was prepared.[103]

Pathogenesis

The mechanisms through which *Campylobacter* produces disease are not fully understood but likely involve three potential mechanisms[104]: (1) adherence to the intestinal mucosa followed by the elaboration of toxin; (2) invasion of the mucosa in the terminal ileum and colon; and (3) "translocation," in which

the organisms penetrate the mucosa and replicate in the lamina propria and mesenteric lymph nodes. The variety of pathogenic mechanisms may account for the spectrum of disease caused by *Campylobacter.* It is also conceivable that different strains or serotypes of *Campylobacter* may demonstrate different pathogenic mechanisms, as is seen with diarrheagenic *E. coli*.

Clinical Manifestations

Campylobacter may cause disease ranging from mild diarrhea to frank dysentery. Typically, patients experience fever and malaise followed by diarrhea, nausea, and abdominal pain that may mimic appendicitis or inflammatory bowel disease. The symptoms usually resolve in less than 1 week. Bacteremia may rarely occur, with some species implicated more often than are others. *Campylobacter* is also known to cause meningitis, abscesses, septic abortions, pancreatitis, and pneumonia. Guillain-Barré syndrome and Reiter's syndrome are documented to occur as sequelae of *Campylobacter* infection. Increasing evidence has implicated *C. jejuni* as the most common antecedent of Guillain-Barré syndrome and the variant form, Miller-Fisher syndrome, a neuropathy associated with ataxia, areflexia, and ophthalmoplegia.[105,106] Although evidence for molecular mimicry is still preliminary, it is likely that peripheral nerves share epitopes with *C. jejuni*; therefore, the immune response initially mounted to attack *C. jejuni* is misdirected to peripheral nerves.[106] After the resolution of symptoms, patients may continue to shed organisms for as long as 7 weeks.

Diagnosis and Treatment

Culture of the organisms, the gold standard for diagnosis, is routinely accomplished in most laboratories if selective media are used and cultures are incubated at 42° C. Because disease caused by *Campylobacter* is usually mild and self-limited, supportive treatment alone should suffice. In cases of severe disease, erythromycin (10 mg/kg [maximum 500 mg] per dose, given every 6 hours for 5 to 7 days) has been recommended.[97] The need for antibiotic therapy has been questioned, based in part on several studies demonstrating a decrease in the duration of excretion of *Campylobacter* after antibiotic treatment but no decrease in the duration of symptoms. In general, in these studies, antimicrobial therapy was begun late in the course of the illness. In a placebo-controlled, double-blind trial, Salazar-Lindo and colleagues[107] demonstrated a shortened duration of illness, from 4.2 to 2.5 days, in patients who received erythromycin by day 4 of their illness. For cases of *Campylobacter* septicemia, gentamicin (1.5 to 2.5 mg/kg per dose, intramuscularly or intravenously, given every 8 hours) is recommended, with chloramphenicol and erythromycin acceptable as alternatives. Tetracycline (250 to 500 mg per dose, intravenously, given every 6 to 12 hours) may be used in patients older than 8 years of age.[97] Ciprofloxacin is an effective alternative agent but is not approved for use in children younger than 18 years of age. Antibiotic treatment is recommended for outbreaks of *Campylobacter* in day-care settings, because treatment has been shown to eliminate fecal shedding of organisms within 48 hours.[104]

Yersinia

Microbiology

The genus *Yersinia* includes the species *Yersinia pestis*, which causes plague; *Yersinia pseudotuberculosis*, known to cause pseudoappendicitis, mesenteric adenitis, and gastroenteritis; and *Yersinia enterocolitica*, recognized with increasing frequency as a cause of bacterial diarrhea. *Yersinia* is a gram-negative, coccoid bacillus that is facultatively anaerobic. It is non-lactose-fermenting and is observed to be motile at temperatures of 25° C but nonmotile at 37° C.

Epidemiology

Yersinia was initially thought to occur with greater frequency in countries with cooler climates but is now recognized to be worldwide in distribution. Although the true incidence and prevalence of this organism are not known, in some areas yersiniosis occurs more frequently than does shigellosis.[108] Outbreaks due to *Yersinia* have been associated with spread through contaminated water and foods, including bean sprouts, tofu, and chocolate milk.[108] Pork has also been implicated as a source, as in the Fulton County, Georgia, outbreak in 1990, in which chitterlings were found to be the vehicle of infection.[109,110] The organism tends to cause disease more frequently in young children, with 24 months the median age in one study.[111] *Yersinia* may also be spread among household contacts. In addition, there may be an increased incidence in the summer months.[111,108]A case control study from Sweden reported that risk factors for acquiring *Y. enterocolitica* in children less than 6 years of age were foods prepared from unprocessed raw pork products and treated sausages. Other factors were the use of pacifiers and contact with domestic animals.[112]

Pathogenesis

Y. enterocolitica constitutes a heterogeneous group of serotypes with many identified virulence factors.[113] *Y. enterocolitica* produces disease in the intestine through an invasive route. After penetrating the mucosal epithelium, primarily in the ileum, organisms replicate in Peyer's patches and accumulate in the mesenteric lymph nodes.[108] Most serotypes produce an enterotoxin similar to the *E. coli* heat-stable toxin but only at temperatures lower than 30° C; therefore, this toxin may *not* have an important role in disease production by *Yersinia* in the human intestine. There is speculation on the role of preformed toxin in causing disease, because toxin may be produced when the organisms are present in refrigerated foods.[108]

The virulence of *Y. enterocolitica* has been shown to be plasmid related. Different serotypes exhibit different degrees of virulence. Serotypes O:3, and O:9 are the ones most frequently associated with diarrheal disease in Europe and Japan, whereas a larger number of serotypes are seen in North America.[113]

Clinical Manifestations

The most frequent clinical syndrome caused by *Y. enterocolitica* is gastroenteritis, which typically affects young children. After an incubation period of 1 to 11 days, patients develop diarrhea, fever, and abdominal pain.[108] A marked increase in the leukocyte count is common. The symptoms usually resolve in 5 to 14 days but have been known to persist for several months. Excretion of organisms occurs for about 6 weeks.[111] Several complications, including appendicitis, have been documented after *Y. enterocolitica* infection. However, in older children and young adults, *Yersinia* is more likely to produce the pseudoappendicular syndrome, in which the signs and symptoms mimic appendicitis.[108] In this same age group, there has also been an association of *Y. enterocolitica* with nonspecific abdominal pain. Radiographic changes in the terminal ileum more often associated with Crohn's disease, namely mucosal thickening and aphthous ulcers, have been seen with yersiniosis in young adults.

Yersinia bacteremia occurs and, despite therapy with appropriate antibiotics, has a case-fatality rate of 34 to 50%. The finding of *Yersinia* in blood from asymptomatic donors, however, makes the possibility of transient bacteremia seem likely as well.[108]

Sequelae of *Yersinia* infection include erythema nodosum and reactive arthropathy; however, these are more commonly seen in adults.[113] This arthropathy tends to involve the weight-bearing joints of the lower extremities and has been noted to occur most often in *Yersinia* patients who carry the histocompatibility antigen HLA-B27.

Diagnosis and Treatment

Yersinia may be cultured with the use of selective media, preferably with "cold enrichment." Despite the best of methods, culture of *Yersinia* may require as long as 4 weeks. In addition to diagnosis by culture, *Yersinia* may also be detected serologically, through the use of agglutinin titers. These measurements appear to be useful only in conjunction with cultures, because agglutinin titers may be affected by a number of factors, including the patient's age, the underlying disease, and previous use of antibiotics and immunosuppressive agents. These titers may also be more useful in Europe and Japan, where infection is caused by a restricted number of serotypes.

Antibiotics have not been proved effective in alleviating symptoms of *Yersinia* or in shortening the period of its excretion.[108] Pai and associates[114] compared the efficacy of trimethoprim-sulfamethoxazole versus placebo in the treatment of *Yersinia* gastroenteritis and found no significant difference. It should be noted, however, that therapy was not begun until near the end of the course of the illness. In cases of severe disease and in patients with underlying illness, treatment is recommended. Trimethoprim-sulfamethoxazole, aminoglycosides, chloramphenicol, and third-generation cephalosporins are generally recommended. Tetracycline and quinolones are alternative choices for adult patients.[97] Gentamicin or chloramphenicol is recommended for treatment of septicemia. Because septicemia may be associated with an iron overload state,[115] cessation of iron therapy is also recommended during infection.

Cholera

Although cholera is a disease rarely encountered in developed countries, it remains an important entity.[116,117] Investigation of the pathogenesis of cholera led to the recognition and understanding of the mechanism of action of cholera toxin, which remains the prototype for bacterial enterotoxins. Cholera is also important, from a therapeutic perspective, in that initial efforts in the use of oral rehydration solutions were carried out in patients with cholera. However, most importantly, on a worldwide basis, cholera continues to be a major public health problem in almost all developing countries.[118] Cholera afflicts both children and adults, and cholera exists as an endemic disease in more than 100 countries. The death rate is highly dependent on the treatment facilities; the highest mortality rates are in Africa, where case-fatality rates have approximated 10%, especially during epidemic attacks. It is likely that cholera as an endemic infection causes 100,000 to 150,000 deaths annually.

Microbiology

Vibrio cholerae is a gram-negative, motile, curved bacillus that is free-living in bodies of salt water. *V. cholerae* is classified on the basis of lipopolysaccharide antigens. Until recently, all epidemic strains of *V. cholerae* were of the O1 serotype. Group O1 is further subdivided into two biotypes: classic and El Tor. Other serotypes were thought to cause sporadic cases of diarrhea but not epidemic disease. This dictum was discarded after the development of an ongoing epidemic in Asia and South America caused by a new serotype, O139, synonym Bengal.[119] Although the pathogenesis and clinical features of O139 cholera are identical to those of O1 cholera, persons having immunity to serotype O1 are not immune to the Bengal serotype. This lack of immunity is primarily a result of the unique O139 cell surface antigen.

Epidemiology

V. cholerae is spread via contamination of food and water supplies. There is no evidence of an animal reservoir, but humans may serve as transient carriers. On rare occasions, humans may chronically carry the organism. Owing to the nature of its spread, persons living in areas with adequate sanitation are at minimal, if any, risk for encountering cholera. Cholera does occur in the United States, but usually as a result of imported food brought back by returning international travelers. Travelers from the United States to endemic areas are at low risk (incidence of 1 per 30,000 travelers).[120] Cholera has also been isolated from oysters in the Gulf Coast.[121] However, owing to the frequency of international travel, it is important for the clinician who encounters a patient with severe cholera symptoms (dehydration and rice-water stools) to suspect this infection even in nonendemic areas.

Pathogenesis

V. cholerae enters its potential host through the oral route, usually in contaminated food or water. Volunteer studies have shown that a relatively large number of organisms (approximately 10^{11}) must be ingested to produce symptoms. Similar to other ingested organisms, *V. cholerae* must survive the acidic gastric environment. The importance of gastric acidity as a host-protective factor is borne out by the increased occurrence of cholera in patients with absent or reduced gastric acidity.

The organisms travel to the small intestine, where they adhere to the epithelium. This process may be aided by production of mucinase. The intestinal epithelium remains intact with normal morphology. *Vibrio* species produce a toxin that is composed of a central subunit (A) surrounded by five B subunits; the latter bind to a ganglioside, GM_1, which serves as the toxin receptor. This binding facilitates the transfer of the A subunit across the cell membrane, where it is cleaved into two components, denoted A_1 and A_2. The disulfide linkage between A_1 and A_2 is reduced to liberate an active A_1 peptide, which acts as a catalyst to facilitate the transfer of adenosine diphosphate-ribose from nicotinamide adenine dinucleotide to a guanyl nucleotide-binding regulatory protein (G_s). G_s then stimulates adenylate cyclase, located on the basolateral membrane, thereby increasing cyclic adenosine monophosphate. This result in turn leads to chloride secretion and a net flux of fluid into the intestinal lumen.

Although this mechanism of toxin action adequately explains the clinical symptoms of cholera, similar symptoms have been noted in patients infected with strains that do *not* produce the classic cholera toxin. This has led to the recognition that *V. cholerae* harbors additional virulence factors in the bacterial genome that may contribute to diarrheal disease and must be considered in the design of a nonreactigenic vaccine.

Newly recognized toxins produced by *V. cholerae* include zonula occludens toxin and the accessory cholera toxin.[122,123]

Clinical Manifestations

After an incubation period, commonly 1 to 3 days, the symptoms of cholera usually begin abruptly with profuse, watery diarrhea and sometimes with vomiting. The stool soon becomes clear, with bits of mucus giving it the so-called rice-water appearance. Patients do not experience tenesmus but rather a sense of relief with defecation. Typically there is no fever. The rate of fluid loss with cholera can be remarkable in severe disease, with purging rates in excess of 1 L/hour reported in adult patients. Despite the dramatic presentation and health risk of "*cholera gravis*," most patients with cholera infection are asymptomatic or experience mild symptoms. In addition to people with reduced gastric acidity, people with blood group O are at increased risk for more severe disease. Other host factors that predispose to increased purging are less clear, but there is great variability in clinical symptoms after infection.

Diagnosis and Treatment

V. cholerae is identified by colonial morphology and pigmentation on selective agar (e.g., thiosulfate citrate bile salt-sucrose agar). Further identification depends on biochemical markers (e.g., positive oxidase reaction) and motility of the organism. Specific serotyping is used to confirm the identification.

The mainstay of cholera treatment is rehydration. In cases in which the disease is less severe and is recognized early, oral rehydration solutions are appropriate and effective. When purging is excessive (more than 10 mL/kg per hour), intravenous rehydration is required.

Antibiotics have been shown to cause a decrease in duration of the diarrhea, total amount of fluid lost, and length of time organisms are excreted. Tetracycline (250 to 500 mg per dose, given every 6 hours for 3 to 5 days) has been recommended as an appropriate antibiotic for adults, and furazolidone (1.25 mg/kg [maximum 100 mg] per dose, given every 6 hours for 10 days) has been suggested for children and pregnant patients. Ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and doxycycline may also be used. Single-dose ciprofloxacin has also been shown to be effective in the treatment of *V. cholerae* O1 or O139,[124] although this drug is not approved for use in children. A recent randomized controlled trial showed that a single dose of azithromycin 20 mg/kg was superior to ciprofloxacin for treating cholera in children.[125]

Despite much progress, an ideal cholera vaccine is not yet available. An ideal vaccine would provide a high level of long-term protection even to those at high risk for severe illness (e.g., people with blood group type O), and this protection would commence shortly after administration of a single oral dose. New oral vaccines have been developed for cholera, including both killed vaccines and live attenuated strains.[126,127] CVD 103-HgR, a vaccine strain with a 94% deletion of the *ctxA*, proved efficacious against experimental challenge with *V. cholerae* El Tor Inaba 3 months after inoculation, suggesting it may be useful for travelers to endemic areas.[128] Unfortunately CVD 103-HgR was not effective in a field trial.[129] Peru-15, a nonmotile strain that colonizes better than CVD 103-HgR, has been shown to be highly effective in volunteer studies.[130] A reformulated bivalent (*V. cholerae* O1 and O139) killed whole cell oral vaccine was also found to be safe and immunogenic in a cholera-endemic area in India.[131] Other live attenuated O1 oral cholera vaccines are in earlier stages of development including VA1.3 vaccine from India, IEM 108 from China,[132] and an intranasal vaccine.[133]

Other Vibrios

The noncholera vibrios, *V. parahaemolyticus, V. pluvialis, V. mimicus, V. hollisae, V. furnissii,* and *V. vulnificans*, have been shown to cause gastrointestinal illness, wound infections, and septicemia.[134] Although each organism has its own characteristics, most noncholera vibrios produce a protein toxin identical to the classic cholera toxin. Some species also produce a heat-stable toxin similar to *E. coli* heat-stable toxin.[135] Although these organisms produce a cholera-like illness, the stool may sometimes contain blood and leukocytes, and sepsis can occur. This has led to speculation that some members of this group, namely *V. parahaemolyticus*, may be capable of invasiveness as well as toxin production.[134] In the United States, gastroenteritis caused by these vibrios is most often associated with the ingestion of raw oysters.[136]

Gastroenteritis caused by non-O1 vibrios tends to be far milder than that caused by *V. cholerae*. In severe cases of diarrhea or septicemia, antibiotics may be helpful, with the agents used for *V. cholerae* recommended.

Escherichia coli

E. coli constitutes a diverse group of organisms, including both nonpathogenic strains, which are among the most common bacteria in the normal flora of the human intestine, and pathogenic strains. Pathogenic *E. coli* strains that cause diarrheal illness have been recognized since the 1940s.[137]

These diarrheagenic *E. coli* have been studied extensively and are currently classified, on the basis of serogrouping or pathogenic mechanisms, into six major groups: (1) enteropathogenic *E. coli* (EPEC), an important cause of diarrhea in infants in developing countries; (2) enterotoxigenic *E. coli* (ETEC), a cause of diarrhea in infants in developing areas of the world and a cause of traveler's diarrhea in adults; (3) enteroinvasive *E. coli* (EIEC), which cause either a watery ETEC-like illness or, less commonly, a dysentery-like illness; (4) Shiga toxin-producing *E. coli* (Stx-producing; formerly known as enterohemorrhagic *E. coli*), which cause hemorrhagic colitis and HUS; (5) enteroaggregative *E. coli* (EAggEC); and (6) diffusely adherent *E. coli* (DAEC), which along with EPEC have been implicated as causes of acute and persistent diarrhea. Each of these groups of *E. coli* has unique properties (Table 39-3).

Enteropathogenic *Escherichia coli*

EPEC is a major cause of diarrhea in developing countries. As much as 30% to 40% of infant diarrhea, particularly in those less than 6 months of age, may be caused by EPEC, and in some studies EPEC infection exceeds that of rotavirus.[138-141] In North America and the United Kingdom, EPEC infections were common during the 1940s through the 1960s; now they are most commonly associated with sporadic cases and nosocomial or day-care outbreaks.[142,143] However, because of the general unavailability of serotyping, the true incidence of EPEC-associated diarrhea may be underestimated. A 1997 study in Seattle children with diarrhea, and a 2005 study in Cincinnati in which DNA probes were used to screen *E. coli* present in stool, found a high incidence of EPEC-like organisms (atypical EPEC) in this population.[144,145]

TABLE 39-3. **Diarrheagenic *Escherichia coli***

Name	Abbreviation	Pathogenic Mechanisms	Illness
Enteropathogenic *E. coli*	EPEC	Adherence to enterocytes	Infantile diarrhea in developing countries
Enterotoxigenic *E. coli*	ETEC	Enterotoxin elaboration	Infantile diarrhea in developing countries; traveler's diarrhea
Enteroinvasive *E. coli*	EIEC	Invasion of epithelial cells; toxin elaboration	Watery diarrhea/dysentery
Stx-producing *E. coli**	Stx	Cytotoxin elaboration Adherence	Hemorrhagic colitis; hemolytic-uremic syndrome
Enteroaggregative *E. coli*	EAggEC	Adherence Enterotoxin elaboration	Persistent diarrhea in developing countries
Diffusely adherent *E. coli*	DAEC	Adherence	Diarrhea

*Formerly enterohemorrhagic *E. coli* (EHEC).

The hallmark of EPEC infection is the "attaching and effacing" lesion seen in the intestine. This lesion is characterized by destruction of microvilli and intimate adherence between the bacterium and the epithelial cell membrane. Directly beneath the surface of the adherent organism, there are marked cytoskeletal changes in the enterocyte, including accumulation of actin polymers. Often, the bacteria are raised on a pedestal-like structure as a result of this actin accumulation. A number of steps are probably responsible for the development of this attaching and effacing lesion. As proposed by Donnenberg and Kaper,[146] EPEC pathogenesis consists of three phases: (1) localized adherence, which brings the bacteria in close contact with the enterocyte (e.g., docking); (2) signal transduction, including increases in intracellular calcium and protein phosphorylation; and (3) intimate adherence, a multigene process encoded in the bacterium by a locus of enterocyte effacement.[147,148] The dramatic loss of absorptive microvilli in the intestine presumably leads to diarrhea via malabsorption. Although this is probably the predominant mechanism, some evidence suggests that a separate secretory mechanism is also involved.

Patients with symptomatic EPEC infection typically experience diarrhea, vomiting, malaise, and fever. The stool may contain mucus but does not usually contain blood. Symptoms with EPEC infection are more severe than with some other enteric infections and may persist for 2 weeks or longer.[137] In some patients, EPEC has caused protracted diarrhea with dehydration, malnutrition, and zinc deficiency as complications; treatment with parenteral hyperalimentation has been required.[143] EPEC can be detected by serotyping of isolated *E. coli*,[142] by demonstration of the presence of the enterocyte adherence factor or other virulence genes using molecular probes,[149] or by identification of the attaching and effacing phenotype using tissue culture cells.[150] These assays are not commonly used in the clinical microbiology laboratory. Diagnosis of EPEC may be made by demonstrating the presence of adherent organisms on small intestinal or rectal biopsy.[142,143]

Although controlled studies of antibiotic therapy for EPEC have been few, the significant morbidity associated with this agent argues for treatment with antibiotics in most cases. Trimethoprim-sulfamethoxazole (trimethoprim, 5 mg/kg [maximum 160 mg], plus sulfamethoxazole, 25 mg/kg [maximum 800 mg] per dose, given every 12 hours) has been used with some success, as have oral neomycin and gentamicin.

Enterotoxigenic *Escherichia coli*

ETEC are recognized as an important cause of diarrhea in infants in developing areas of the world. In endemic areas, children in the first few years of life may be infected several times each year. It is an important cause of diarrhea in infants and in travelers from developed to undeveloped countries, especially in regions of poor sanitation.[151] In the United States, cases of ETEC among children are uncommon. ETEC is also a major cause of traveler's diarrhea in adults. Fecal-oral transmission and consumption of heavily contaminated food or water are the most common vehicles for ETEC infection. The prevention of the spread of ETEC depends on ensuring appropriate sanitary measures: handwashing and proper preparation of food, chlorination of water supplies, and appropriate sewage treatment and disposal.[151]

The production of disease by ETEC begins with colonization of the small intestine. There the bacteria depend on fimbriae (also called *pili*) to facilitate attachment to the mucosal surface and overcome the forward motion of peristalsis. This attachment process causes no detectable structural changes in the architecture of the brush border membrane but does allow the bacteria to release their enterotoxins, heat-labile toxin (LT) and heat-stable toxin (ST), in close proximity to the enterocyte brush border membrane where toxin receptors are present.[152] These toxins in turn stimulate adenylate cyclase (in the case of LT) or guanylate cyclase (in the case of ST), and both ultimately result in a net fluid secretion from the intestine (see the reviews by Cohen and Giannella[153] and by Sears and Kaper[154]). Two endogenous ligands for the ST receptor, guanylin and uroguanylin, have been identified.[155,156] This discovery is consistent with the hypothesis that ST is a superagonist and exerts its diarrheal action by means of usurping a normal secretory mechanisms in the intestine (e.g., by molecular mimicry of these less potent endogenous ligands). Uroguanylin may also act as a hormone regulating salt and water excretion in the kidney in response to an oral salt load.[157]

Clinically, ETEC infection causes nausea, abdominal pain, and watery diarrhea. Stools typically contain neither mucus nor leukocytes. ETEC can be diagnosed with the use of bioassays such as the suckling mouse assay, immunoassays, or gene probes specific for either ST or LT. PCR assays are also available. However, none of these assays is commonly used in the clinical microbiology laboratory. Supportive measures are sufficient therapy for most cases of ETEC diarrhea, with oral rehydration a mainstay of therapy. Antibiotics, including trimethoprim-sulfamethoxazole, have been shown to decrease the duration of fecal excretion of the organisms. Quinolone antibiotics may be more effective,[158] but they are not recommended for use in children. Rifaximin was also shown to provide protection against and treatment for travelers' diarrhea.[159-161]

Cholera toxin (CT) is more than 80% homologous to LT, and vaccination with CT-B subunit (CT-B) based vaccines elicits a protective immune response against LT-producing ETEC strains. Peru-15 (an oral live attenuated candidate cholera

vaccine) has been engineered to express and secrete high levels of CT-B; this candidate vaccine Peru-15pCTB has promising characteristics of an oral, single-dose, bivalent cholera/ETEC vaccine[162] and is currently undergoing Phase 1 clinical trial.

Enteroinvasive *Escherichia coli*

EIEC share many common features, including virulence mechanisms, with *Shigella*. These organisms preferentially colonize the colon and invade and replicate within epithelial cells, where they cause cell death.[137] In addition, both organisms elaborate one or more secretory enterotoxins. Clinically, both *Shigella* and EIEC infections are characterized by a period of watery diarrhea that precedes the onset of dysentery (scanty stools containing mucus, pus, and blood). More commonly, in contrast to *Shigella*, only this first phase of watery diarrhea is seen in EIEC infection. This illness is clinically indistinguishable from other causes of bacterial diarrhea (e.g., ETEC) or nonbacterial infectious diarrhea. In a minority of patients with EIEC infections, the dysentery syndrome of characteristic stools, tenesmus, and fever is also seen. Bacteremia is not reported.

Infection due to EIEC is uncommon, but foodborne outbreaks of disease have occurred in the United States and aboard cruise ships. Diagnosis is dependent on bioassay (the Sereny test), serotyping, ELISA, or DNA probe techniques. None of these tests is commonly available in the clinical laboratory. Treatment is currently limited to supportive measures, although ampicillin given intramuscularly has been associated with bacteriologic cure and clinical improvement.

Shiga Toxin-Producing *Escherichia coli*

Stx-producing *E. coli* are a distinct class of organisms that have been identified since 1983 as the cause of two recognizable syndromes: hemorrhagic colitis and HUS.[163,164] Hemorrhagic colitis is an illness characterized by crampy abdominal pain, initial watery diarrhea, and subsequent development of grossly bloody diarrhea with little or no fever. Although there may be more than 100 serotypes in this class of diarrheagenic *E. coli*, in North America the *E. coli* serotype O157:H7 is the prototypic member of this family of organisms. *E. coli* O157:H7 is the most common cause of infectious bloody diarrhea in the United States.[165] Similarly, HUS, which is defined as the triad of acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, is also highly associated with antecedent *E. coli* O157:H7 infection.

Stx-producing *E. coli* infections may occur in sporadic cases, but they have also been associated with outbreaks of disease in nursing homes, day-care centers, and other institutions; several reviews have been published.[166-169] It is estimated that *E. coli* O157:H7 causes approximately 10,000 to 20,000 infections per year in the United States alone and may be responsible for 250 deaths annually.[170] Inadequately cooked hamburgers were most likely the source of the first outbreak and remain the most common vehicle of transmission. In 1993 there was a large epidemic in the western United States; inadequately cooked hamburgers were again implicated as the cause. Aside from ground beef, many other food vectors have been implicated. Epidemics have been attributed to apple juice or cider, and large-scale outbreaks in Japan have been associated with bean sprouts. Contaminated water has also been a source of infection.[171,172] Common to all of these outbreaks is a reservoir of Stx-producing *E. coli* in the intestines of cattle and other animals that are asymptomatic. Infection is spread either by direct contact with intestinal contents or through droppings or water runoff from contaminated pastures. A low infectious dose for Stx-producing *E. coli* and the resistance of these organisms to gastric acid and to the food preserving process (high salt and drying) contribute to the high attack rate. The low infectious dose also contributes to frequent person-to-person transmission.[166-169] Nonfoodborne outbreaks have been associated with attending child day care,[173] drinking contaminated water,[174] and swimming in unchlorinated water.[175]

Both the very old and the very young appear to be at increased risk for Stx-producing *E. coli* infection and its complications.[166-169] Clinical features and complications of *E. coli* O157:H7 infection include bloody diarrhea, nonbloody diarrhea, HUS, thrombotic thrombocytopenic purpura, and, uncommonly, asymptomatic infection.[166] Symptoms may persist for several days or, less commonly, for several weeks. Early reports suggested that carriage of the organism was brief and that prompt culture was necessary to recover these organisms.[176,177] More recently, prolonged shedding has been observed.[173,178] This has led to the recommendation that two negative stool cultures be obtained before a child is allowed to return to day care.[173]

The identification of Stx-producing *E. coli* is made difficult because it is not possible to differentiate disease-producing *E. coli* from normal enteric flora on the basis of standard microbiologic techniques. There are currently six techniques for identification of Stx-producing *E. coli*: biochemical markers with serotyping (most commonly used), serum antibody tests, cytotoxin bioassays, DNA hybridization, PCR-based tests, and cytotoxin detection (including ELISAs). Some of these methods (e.g., toxin-based assays) detect the presence of cytotoxin-producing organisms, including non-O157 serotypes. It may be important to use both biochemical markers and toxin-based assays in clinical practice to identify organisms that are truly pathogenic.[179] The increased use of non-culture-based methods, such as Shiga toxin enzyme immunoassays, has resulted in a dramatic increase in reports of non-O157 Stx-producing *E. coli*.

Prevention of disease transmission is made difficult by the fact that these organisms colonize the intestine of healthy cattle and other food animals, including beef, pork, lamb, and poultry. Therefore, they can survive and multiply in the food chain. Proper cooking destroys these organisms; in hamburgers, an internal cooking temperature of 70° C (157° F) renders the meat safe. Practically, safe cooking most commonly results in a gray hamburger (not pink), with clear juices. Risk can be lowered by educating consumers about cross-contamination, use of warning labels now affixed to meat in the United States, and improvements in meat processing and microbial contamination detection.

At present there is no effective therapy to treat Stx-producing *E. coli* disease, so prevention is the most important strategy. Hemorrhagic colitis has been confused with a number of other conditions, including ischemic colitis, appendicitis, Crohn's disease, ulcerative colitis, cecal polyp, pseudomembranous colitis, and an acute abdomen (ileitis). Therefore, an important aspect of treatment of Stx-associated hemorrhagic colitis is making the correct diagnosis and avoiding unnecessary diagnostic studies such as angiography and laparotomy. The mainstay of therapy for hemorrhagic colitis is the management of dehydration, electrolyte abnormalities, and gastrointestinal blood loss. Antimicrobial agents may help by killing the bacterial pathogens, but they may also cause harm by increasing the

release and subsequent absorption of Stx.[180] Trials of antibiotic treatment of Stx-producing *E. coli* infection are inconclusive. Although these organisms are uniformly sensitive to antimicrobials in vitro, at present there is no evidence that antimicrobial therapy is helpful in diminishing the severity of illness, shortening the duration of fecal excretion, or preventing HUS.[181] Of greater concern is a study suggesting an increased incidence of HUS in those treated with antimicrobials.[182] An attempt to assimilate findings of published series on the subject via a meta-analysis failed to identify an increased risk of HUS in those treated with antimicrobials.[183] Regardless, until more data are available on this topic, most experts would agree that treatment of Stx-producing *E. coli* with antimicrobials is not advisable.[184] Rifaximin does not cause replication of phage or strain lysis and therefore might not increase the risk of HUS, but it has not been studied in humans with O157 infection.[185,186] A multicenter trial failed to demonstrate an improved clinical course in pediatric patients treated with Stx-binding resin.[223] Other toxin neutralizing therapies are currently under investigation, including the use of G3 receptor analogues and monoclonal antibodies.[186,187]

Enteroaggregative and Diffusely Adherent *Escherichia coli*

EAggEc and DAEC were initially categorized as part of a larger group of enteroadherent *E. coli*. These strains differed from classical EPEC strains in that they did not show localized adherence in the Hep-2 cell assay.[188] The aggregative or "stacked brick" appearance of EAggEC in this bioassay permitted epidemiologic investigation, and EAggEc were found to be associated with persistent diarrhea in developing counties. There was uncertainty about EAggEc pathogenicity because these organisms are found in apparently healthy individuals and because some epidemiologic studies failed to show an association with disease.[189,190] However, evidence from volunteer studies[191,192] and outbreaks[193] has confirmed the pathogenicity of some EAggEC strains. Studies at Cincinnati Children's Hospital Medical Center have shown that EAggEC are an important unrecognized cause of acute infant diarrhea.[145] The mechanisms by which these organisms cause disease is thought to involve adherence to the intestinal mucosa including dispersin protein, a newly identified EAggEC virulence factor, followed by secretion of one or more enterotoxins and/or stimulation of IL-8 release by a flagellar protein.[194-196] DAEC are less well characterized but have also been associated with diarrheal disease. Both the HEp-2 cell assay and DNA probes have been used to identify these organisms, but these are not routinely available in the clinical microbiology laboratory.

Clostridium difficile

C. difficile is a spore forming gram-positive anaerobic bacillus. Disease caused by this organism can manifest in a variety of ways, ranging from asymptomatic carriage to potentially life-threatening pseudomembranous colitis. It is a frequent cause of antibiotic-associated diarrhea and a common nosocomial pathogen.

Epidemiology

Of great interest in the study of *C. difficile* is the difference in the incidence of isolation of the organism and its toxin in various age groups. *C. difficile* toxin has been found in the feces of 10% of normal-term neonates and 55% of those in a neonatal intensive care unit.[197] Most infants found to have toxin in their stools are asymptomatic. A small group of toxin-positive infants have signs and symptoms of necrotizing enterocolitis, but no clear relation to *C. difficile* or its toxin has been demonstrated. The presence of *C. difficile* toxin in these asymptomatic infants may indicate the coexistence of some protective antitoxic substance[198] or may reflect a lack of appropriate toxin receptors in patients in this age group.[199]

The incidence of *C. difficile* toxin positivity decreases beyond the neonatal period. The incidence of asymptomatic carriage in children older than 2 years of age approaches that in healthy adults (about 3%). Furthermore, not all of these organisms are toxin producers. Adults who develop disease from *C. difficile* infection are also more likely than children to experience severe colitis symptoms, although there are some reports of severe infection in infants, especially those with underlying intestinal pathology such as patients with Hirschsprung's disease or necrotizing enterocolitis.[200]

Beginning in December 2002, outbreaks of an unexpectedly large number of *C. difficile* cases were reported in Quebec, Canada.[201] These outbreaks were characterized by a 4.5-fold increased prevalence over historical incidence rates, a 5-fold increase in mortality, and a 2.5-fold increase in the proportion of complicated cases. During the outbreaks in Canada, a new hypervirulent strain, identified as BI/NAP1/027, was found to be responsible for the increased prevalence and severity.[201] All pathogenic strains of *C. difficile* produce toxin A or B or both. This BI/NAP1/027 hypervirulent strain produces 16 times more toxin A and 23 times more toxin B than other strains and has now been found throughout the United States and in many parts of the world. Although rates of *C. difficile* infection are increasing coincidentally with this new strain, it does not appear that the increased prevalence is predominantly due to the emergence of the BI/NAP1/027 strain. Non-BI/NAP1/027 community-acquired strains appear to be more important to the overall increased disease burden. During 2005, nonhypervirulent strains caused severe disease in generally healthy persons in the community at a rate of 7.6 cases per 100,000 population without the usual risk factors of older age, exposure to health care facilities, or antimicrobial use.[202] A 5-year retrospective study revealed an increase in the number of patients seen in the emergency department with community-acquired *C. difficile* infection.[203] A recent prospective cohort study found *C. difficile* toxin in 6.7% of stool samples tested in children seen for diarrhea in a children's hospital emergency department in Seattle; this rate is may be an underestimate because in this study only toxin B positive strains were identified.[204] A similar incidence of community-acquired *C. difficile* associated diarrhea was found in Connecticut.[205]

Pathogenesis

C. difficile elaborates two important toxins responsible for the inflammation, fluid, and mucus secretion as well as damage to the intestinal mucosa. Toxin A, which is responsible for the activation and recruitment of inflammatory mediators, is a large protein (308 kDa) that binds to an enterocyte surface receptor and activates an intracellular G protein-dependent signal transduction mechanism.[206] Bound toxin results in altered permeability, inhibition of protein synthesis, and direct cytotoxicity. Toxin B demonstrates cytotoxic effects. Most strains produce both toxins, but there are some that elaborate only one or

none.[207] A third "binary toxin" or cytodistending toxin (CDT) (actin-specific ADP-ribosyltransferase) is found in 1 to 16% of infected patients and may be associated with more severe diarrhea. Binary toxin has enterotoxic activity in vitro, but its role, if any, in the pathogenesis of *C. difficile* infection is not yet clear. It may act synergistically with toxins A and B in causing severe colitis.[208]

The ability to form spores is thought to be a key feature in enabling the bacteria to persist in patients and the physical environment for long periods, thereby facilitating its transmission. *C. difficile* is transmitted through the fecal-oral route. Postulated risk factors include contact with a contaminated health care environment, contact with persons who are infected with and shedding *C. difficile*, and ingestion of contaminated food.[205] Some studies reveal increase risk in patients on gastric acid-suppressing medication.[207]

Clinical Manifestations

Most patients experience mild, watery diarrhea; abdominal pain and/or tenderness may be present. Although symptoms often last only a few days and spontaneously resolve, in some patients, symptoms persist for weeks to months. There is a broad range of symptoms ranging from asymptomatic carrier, mild to moderate diarrhea with or without blood, colitis with mucopus, and, less frequently, pseudomembranous enterocolitis, where patients are often extremely ill, with high fever, leukocytosis, and hypoalbuminemia. Any mucosal disease, including inflammatory bowel disease, is thought to be a risk factor. In children, inflammatory bowel disease is associated with increased prevalence of *C. difficile* infection.[209] *C. difficile*–related diarrhea frequently occurs in the setting of antimicrobial administration, and hospitalization is another major risk factor for the acquisition of infection.

Diagnosis and Treatment

C. difficile should be suspected in cases of colitis or mild diarrhea in which blood and leukocytes are noted in the stools. Concurrent or recent exposure (within several weeks) to antibiotics should increase the suspicion for *C. difficile* as the causative agent. The use of virtually any antibiotic may predispose to *C. difficile* disease.

The "gold standard" for diagnosis of *C. difficile* is the detection of toxin from fecal samples, using a cell cytotoxicity neutralization assay, and it is based on identifying *C. difficile* toxin B in cell culture. This assay has a high sensitivity and specificity, but it can take up to 48 hours. Stool culture requires specialized laboratory technique, and it will identify organisms that are not toxin producers, making interpreting a stool culture a challenge. Enzyme immunoassays can detect toxins A and/or B, are rapid, and are less expensive, with a turnaround time of a few hours. They have high specificity, but sensitivity is between 65 to 85% because of the high level of toxin that needs to be present.[207] Sigmoidoscopy in cases of pseudomembranous colitis typically reveals friable white exudate overlying multiple ulcerated areas. The histologic findings of such lesions are depicted in Figure 39-4. Less commonly, pseudomembranes may not be present in the rectosigmoid but may be present in the more proximal colon.

Pending additional data, for now it seems prudent to restrict routine testing for *C. difficile* in children with appropriate symptoms who are younger than 12 months to those with unusual risk factors and to test children between 1 and 2 years of age with appropriate symptoms and antimicrobial exposure. Children older than 2 years of age should be evaluated in the same manner as older children and adults, and infection should be considered even in the absence of prior antimicrobial exposure.[200]

In cases of mild diarrheal illness caused by *C. difficile*, discontinuation of any antibiotics the patient is receiving may be sufficient therapy. Although vancomycin is the only U.S. FDA-approved drug for treatment of *C. difficile* infection, oral metronidazole remains the first-line therapy for mild infection. Compared with vancomycin, metronidazole is much less expensive and has similar efficacy, but in severe infection vancomycin is more effective.[208] In cases of severe illness and especially in cases of pseudomembranous colitis, treatment should include oral vancomycin.

There is a fairly high rate of relapse of illness, generally 15 to 20%, after treatment of *C. difficile*. These relapses usually occur within 1 month of completion of therapy and sometimes but not always result from the activation of *C. difficile* spores remaining from the primary infection.[206] Most of these cases of

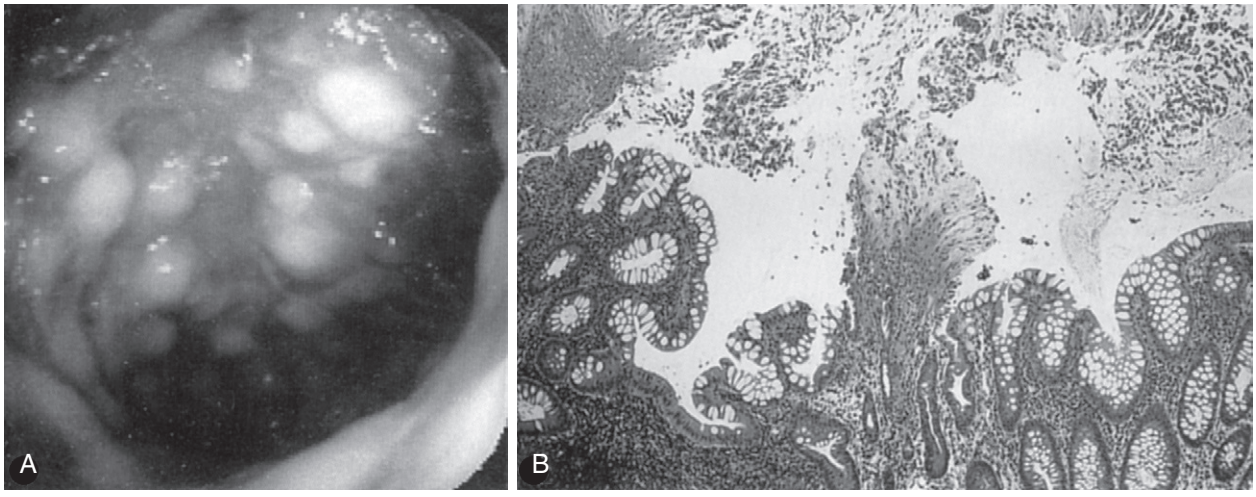

Figure 39-4. (**A**) The endoscopic appearance of the sigmoid colon with multiple densely adherent plaques (pseudomembranes). (**B**) Mucosal biopsy shows a focus of necrotizing enterocolitis with a typical volcano lesion (accumulated fibropurulent exudate intermixed with mucus). From Bates M, Bove K, Cohen MB. Pseudomembranous colitis caused by *C. difficile*. J Pediatr 1997;130:146, with permission.

relapse are responsive to a second course of metronidazole or vancomycin. The first relapse episode can be treated with the same agent that was used for the initial episode. For the second recurrence, vancomycin or a vancomycin taper or pulse therapy has been recommended. Recurrences can be multiple, and recurrent *C. difficile* treatment is a challenge. Other treatment options include alternative antibiotics such as rifaximin in conjunction with vancomycin[210] or nitazoxanide.[211] *Lactobacillus* GG and *Saccharomyces boulardii* have been beneficial for the prevention of antibiotic-associated diarrhea.[212] For treatment of *C. difficile* diarrhea recurrence, *S. boulardii* was found to be effective in adults but has not been well studied in children. Other therapies for recurrent infection include fecal transplantation[213] and intravenous immunoglobulin.[214] None of the toxin binding agents are currently proven to be effective.[215]

Aeromonas and Plesiomonas

Several organisms not previously recognized as enteric pathogens have been linked to diarrheal disease. This includes organisms of the genus *Aeromonas* and the closely related bacterium *Plesiomonas shigelloides* (previously classified as *Aeromonas shigelloides*). These organisms are gram-negative, facultatively anaerobic bacilli classified in the family Vibrionaceae. They are oxidase-positive, differentiating them from members of the Enterobacteriaceae.[216]

Aeromonas

Several members of the genus *Aeromonas*, including *Aeromonas hydrophila,* are common inhabitants of fresh and brackish water in the United States. These organisms were initially recognized as opportunistic pathogens in immunocompromised hosts, especially those with malignant hematologic diseases. The organisms also have been known to cause disease in patients with underlying hepatobiliary disease.[216] *Aeromonas* has been isolated from healthy persons as well and has therefore been thought to be part of the normal flora. Despite initial studies that yielded conflicting results,[217] it is now generally accepted that *A. hydrophila* is an enteric pathogen.

Studies in Australian children with diarrhea have found *Aeromonas* species present in 10% of patients.[218] Infection appears to occur most frequently in children younger than 2 years of age.[219] Of patients with *Aeromonas* isolated from stool cultures at Cincinnati Children's Hospital Medical Center, approximately 50% were younger than 3 months. *Aeromonas* infection is also seasonal, occurring more often in the summer months.[216]

Not all *Aeromonas* species are pathogenic. In a prospective control study of children with diarrhea, *Aeromonas* was isolated only from control subjects.[145] The method of pathogenesis remains unclear. Both cytotoxic[219] and enterotoxic[216] properties have been observed, but neither these nor other pathogenic mechanisms are found consistently in strains isolated from patients with *Aeromonas*-associated disease.[217] *Aeromonas caviae*, a commonly isolated species, demonstrates both adherence and cytotoxin production.[220]

Clinical symptoms attributed to *Aeromonas* can be grouped into three categories: (1) acute watery diarrhea, the most common syndrome; (2) dysentery, which usually is self-limited; and (3) persistent watery diarrhea. Cramping abdominal pain and vomiting may also occur.[219] Symptoms may occasionally be severe and, especially when dysentery is present, have been incorrectly diagnosed as ulcerative colitis.[218]

In mild cases of *Aeromonas* infection, supportive treatment should suffice. In patients who are immunocompromised, are otherwise acutely ill, or have persistent illness, treatment with antibiotics is recommended. Trimethoprim-sulfamethoxazole is usually effective (trimethoprim, 5 mg/kg [maximum 160 mg], plus sulfamethoxazole, 25 mg/kg [maximum 800 mg] per dose, given every 12 hours for 14 days), as are tetracycline, chloramphenicol, and the aminoglycosides.[216] Most strains of *Aeromonas* are resistant to the penicillins, including ampicillin.[216]

Plesiomonas

P. shigelloides, like *Aeromonas*, is commonly found in the environment,[221] especially in bodies of water, including water from a home aquarium.[222] Unlike *Aeromonas*, however, *Plesiomonas* has been reported to occur in epidemics, with contaminated water often found to be the cause.[221] *Plesiomonas* is also known to be spread through improperly cooked seafood.[223]

The pathogenesis of disease caused by *P. shigelloides* is not well understood. A cytotoxin has been found in some strains[221] but not in others. An invasive mechanism is also suspected, because of the colitis symptoms.[223] In addition to small-volume stools with leukocytes and possible blood, patients may also experience severe abdominal pain. Fever has been seen in approximately one third of patients.[223] In one group of adult patients, symptoms persisted longer than 2 weeks in 75% and longer than 4 weeks in 32%.[223]

Diagnosis of *P. shigelloides* is made by stool culture. Although this illness is usually self-limited, treatment with antimicrobial agents has been shown to decrease the duration of symptoms,[223] with trimethoprim-sulfamethoxazole or aminoglycosides suggested as appropriate choices. There are no controlled trials of antimicrobial treatment of gastroenteritis caused by this organism.

Mycobacterium avium-intracellulare

Mycobacterium avium and *Mycobacterium intracellulare*, known collectively as *Mycobacterium avium-intracellulare* or *Mycobacterium avium* complex (MAC), are acid-fast bacilli that have been recognized primarily for their role in cases of atypical tuberculosis. These organisms are now recognized as causative agents of diarrheal symptoms as well. In a review of pediatric cases of atypical mycobacterial infections, Lincoln and Gilbert[224] described two immunocompetent patients whose clinical findings included diarrhea and colonic ulceration.

Of even greater significance than these sporadic cases of MAC infection in immunocompetent hosts is its occurrence among immunocompromised patients. In patients with the acquired immunodeficiency syndrome, MAC is among the most commonly isolated agents causing systemic bacterial infections.[225] These patients may also have chronic diarrhea and abdominal pain.[226,227] MAC has also been noted to cause diarrhea in patients undergoing bone marrow transplantation[228] and in a patient with cystic fibrosis.[229]

The MAC organisms may be cultured from gastric and duodenal aspirates obtained endoscopically and from the stool, the bone marrow, and the blood.[225] Endoscopic examination in patients with MAC may reveal findings similar to those seen in Whipple's disease, with minute superficial ulcerations in the small bowel.[227] Treatment of MAC infections with conventional

antituberculosis agents usually is unsuccessful in eradicating the organisms or alleviating symptoms.[225]

POTENTIAL DIARRHEAGENIC ORGANISMS

Enterotoxigenic Bacteroides fragilis

Bacteroides fragilis is an anaerobic organism that is commonly isolated from normal stool flora. However, some investigators have identified a toxin-producing variant that is enteropathogenic. Enterotoxigenic *Bacteroides fragilis* (ETBF) organisms have been isolated from both healthy persons and those with diarrhea.[230] The only known virulence factor of ETBF is the *B. fragilis* toxin that stimulates secretion of the proinflammatory cytokine, IL-8.[231] Epidemiologic associations with diarrhea in children have been shown for ETBF in several studies[232-235] but not others.[236]

A recent observational study in Bangladesh followed children more than 1 year of age and adults to identify individuals infected with *B. fragilis*.[231] A total of 1209 patients with diarrhea were screened, and 417 (34.5%) yielded *B. fragilis*, of which 86 (7%) were ETBF. The clinical presentation of infection included abdominal pain, tenesmus, and nocturnal diarrhea that lasted a median of 3 days and resulted in dehydration in 14% of individuals. Fecal leukocytes, lactoferrin, and proinflammatory cytokines increased in the ETBF-infected patients.

Brachyspira aalborgi

Intestinal spirochetosis, or the colonization of the large bowel by *Brachyspira aalborgi* and related spirochetes, has been implicated as a cause of diarrhea,[237] but its clinical relevance is still controversial.[238,239] Some studies have shown an association between this organism and bloody diarrhea,[240] although asymptomatic colonization have also been reported.[241] A recent study assessed adult patients with chronic watery diarrhea; of 1174 patients, only 8 were positive for intestinal spirochetosis, it was not diagnosed in the controls (n = 104), and histological resolution of the infection with metronidazole paralleled clinical recovery in 6 patients.[242] The potential of this organism to cause diarrhea requires further evaluation.

Hafnia alvei

This organism has been associated with diarrhea in sporadic cases and in at least one hospital outbreak. Although a causal relation between *Hafnia alvei* and diarrhea has not been clearly established, a subset of this organism may be enteropathogenic. Organisms isolated from patients with diarrhea typically demonstrate the attaching and effacing lesion seen with EPEC, whereas nonpathogenic isolates do not show this characteristic.[243]

Listeria monocytogenes

Invasive illness caused by *Listeria* is well known, but it was only recently that convincing evidence was obtained that *Listeria* can cause acute, self-limited, febrile gastroenteritis in healthy persons. At least seven outbreaks of foodborne gastroenteritis for which *L. monocytogenes* was the most likely etiology have been described.[244] Convincing evidence came from an outbreak of febrile gastroenteritis associated with the consumption of contaminated chocolate milk.[245]

Commonly reported symptoms from outbreaks are fever, diarrhea, arthromyalgia, and headache. Diarrhea is typically nonbloody and watery. Fatigue and sleepiness is frequently reported after an incubation period of 24 hours or less. This gastrointestinal infection is typically self-limited without serious complications in healthy individuals with symptoms lasting 1 to 3 days. A wide variety of foods have been implicated including rice salad, corn-and-tuna salad, chocolate milk, cold smoked rainbow trout, corned beef, cheese, and cold cuts.[244] No data exist regarding the efficacy of treatment with antimicrobials in this illness, and it is not warranted in most instances.

CONCLUSION

Despite this chapter's extensive catalog of both bacterial and viral infectious agents, from 40 to 60% of cases of diarrhea are currently not attributable to any known cause. Undoubtedly, as techniques for identification and culture become more sophisticated, other causative agents will be identified and the percentage of diarrheal illnesses described as idiopathic or nonspecific will continue to decline. Advances in the widespread use of improved oral rehydration solutions have led to a decline in the morbidity and mortality associated with diarrhea. Future advances in preventive measures, including vaccines, may lead to a reduction of the incidence of diarrheal disease.

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