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Introduction

Diarrhea is the result of an imbalance of ion and water movement through the intestinal epithelium. In normal conditions, the intestine absorbs 8–9 l of fluid while only 100–200 ml are excreted in the stools every day. In addition, the intestine absorbs nutrients while simultaneously forming a selective barrier to dangerous substances and pathogenic bacteria. Finally, the intestine is colonized with a wide community of bacteria that exert many beneficial functions, the so-called gut microbiota. Resident commensal and foreign bacteria interact intimately with the gut epithelium and affect host cellular and innate immune responses [1]. Enteric pathogens may hamper the physiological handling of electrolytes and water transport processes by secreting toxins that disturb the function and/or the structure of the intestinal epithelium.

A number of cellular and molecular mechanisms are implicated in infectious diarrhea induced by bacteria, viruses, and parasites. Pathogen-specific virulence factors affect a wide range of cell functions such as ion absorption and secretion, barrier function, and membrane-trafficking pathways causing fluid accumulation in the intestinal lumen.

Several bacterial enterotoxins are capable to increase Cl^- secretion and to reduce Na^+ absorption acting on apical membrane-located transporters or on the lateral spaces between cells, regulated by tight junctions (TJs); other pathogens induce cell damage targeting the cytoskeletal network which is directly implicated in paracellular fluid absorption. Invasive pathogens cause an inflammatory diarrhea characterized by an increase in polymorphonuclear cells (PMNs) in the lamina propria leading to an excess of cytokine secretion and activation of enteric nerves via neuropeptides, eventually resulting in dysenteric diarrhea. Furthermore, a peculiar class of *Escherichia coli*, the enteropathogenic *E. coli* (EPEC),

induces diarrhea through damaging the apical enterocytes, thereby reducing the intestinal absorptive surface. Whatever the mechanism, diarrhea is the manifestation of an altered movement of ions and water that follows an osmotic gradient. The pathophysiology of diarrhea is summarized in Fig. 15.1.

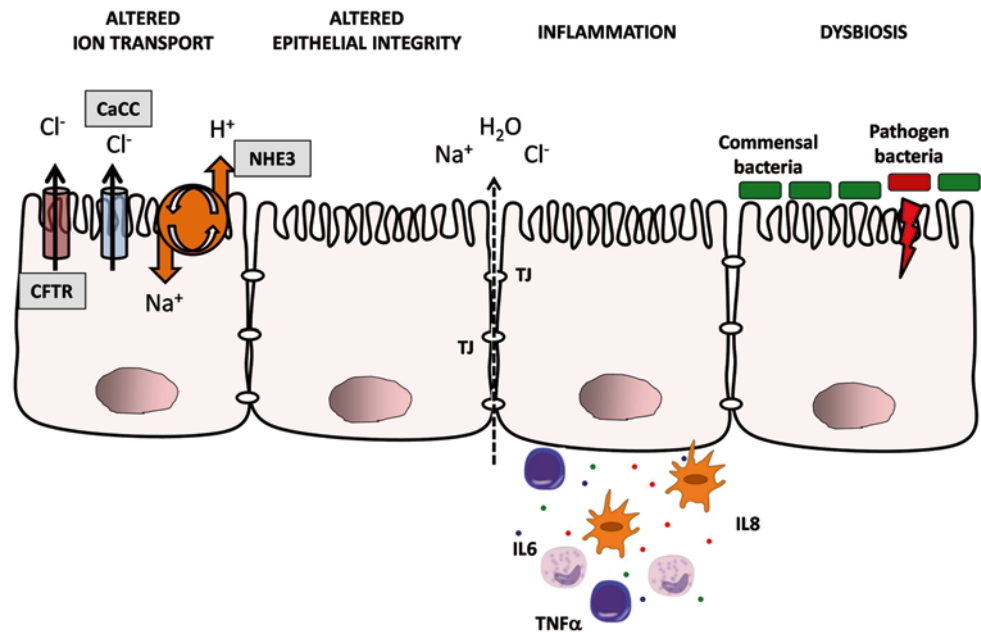
Intestinal Ion Transport and Barrier Functions

In normal conditions, fluid transport across the intestinal epithelial cells is a finely balanced process with fluid absorption predominating on fluid secretion. Electrolyte absorption by apical enterocyte depends on the electroneutral and the electrogenic absorption [2], which involves Na^+ uptake by the Na^+ , H^+ -exchanger (NHE) and Cl^- uptake by the Cl^- , HCO_3^{2-} exchangers. There are several exchangers: NHE1 is located on the basolateral surface of the enterocyte, whereas NHE2 and NHE3 have an apical localization. The relative contribution of NHE2 and NHE3 depends on their localization within the intestine with NHE3 being the main mediator of electroneutral Na^+ uptake [3]. The electrogenic ion absorption is the second major source of apical ion absorption through specific channels. Epithelial sodium channel (ENaC) is the most prominent apical Na^+ channel in the colon and allows Na^+ influx into the cell along its electrochemical gradient [3].

A basal level of fluid secretion is necessary for accomplishing the nutrient digestive functions. The cyclic AMP-dependent chloride channel defined as the cystic fibrosis transmembrane conductance regulator or CFTR is located on the brush border. This channel is responsible for water secretion in basal conditions and under active stimulation by secretagogues [4]. In the intestine, water secretion is a passive process driven by active ion secretion, predominantly by Cl^- secretion [5]. Chloride is taken up across the basolateral membrane via $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter type 1 (NKCC1), as an electroneutral process. Chloride accumulation is a passive process driven by Na^+ concentration

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Fig. 15.1 General mechanisms causing diarrhea. Bacterial toxins can affect intestinal epithelial functions through different mechanisms. Chloride secretion and water secretion depend on the altered functionality of CFTR, CaCC, and NHE channels. Alterations in TJ structures alter the electric gradient causing the movement of both ions and water. Pathogen bacteria induce inflammation through the recruitment of immunity cells and the upregulation of pro-inflammatory cytokines. Finally, an altered gut microbiome composition (dysbiosis) supports pathogen infections. CFTR cystic fibrosis transmembrane conductance regulator, CaCC calcium-activated chloride channels, NHE Na⁺, H⁺-exchanger, TNF α tumor necrosis factor- α , IL interleukin



gradient which is maintained by the basolateral Na, K-ATPase. Two distinct potassium channels are located into the basolateral membrane allowing for potassium recycling thus preventing cellular depolarization, ultimately preserving the electrical driving force for chloride exit from the cell. Therefore, chloride accumulates until apical chloride channels are opened. The bulk of chloride output occurs via cyclic adenosine monophosphate (cAMP)-dependent CFTR chloride channel. However, there is an additional class of chloride channels, the calcium-activated chloride channels (CaCC), that are expressed in the enterocyte apical membrane. These channels drive chloride secretion in response to agonists of cytosolic calcium [4]. Three main intracellular signal-transduction pathways regulate water and electrolyte fluxes across the intestinal mucosa: cAMP; cyclic guanosine monophosphate (cGMP); and calcium-dependent pathways. Recently, a fourth pathway involving nitric oxide (NO) has also been described. Physiological cAMP and cGMP concentrations activate CFTR resulting in a mild secretion of chloride, whereas abnormal CFTR activation in crypt cells results in the secretory diarrhea. cGMP generally results in a more potent, though shorter, chloride secretion than that induced by cAMP [6].

The most common mechanism of bacterial diarrhea is the release of bacterial toxins. Bacterial enterotoxins activate signaling molecules such as cyclic AMP, cyclic GMP, or intracellular Ca²⁺, which, in turn, open cellular Cl⁻ channels leading to an increase in Cl⁻ secretion and consequently of water [5]. The intestinal epithelium forms a selective barrier between the lumen (the external environment) and the body. The electrochemical gradients also depend on TJ that provide a barrier necessary for an efficient transcellular transport. The TJ also support a polarized distribution of

membrane proteins on the basal and apical compartments of epithelial cells [7]. The plasma membranes of adjacent intestinal epithelial cells are linked through the TJ, where claudins, zonula occludens 1 (ZO1), occludin, and F-actin interact. E-cadherin, alpha-catenin 1, beta-catenin, catenin delta1, and F-actin interact to form the adherens junction [8]. The loss of barrier function as a consequence of TJ disruption impairs the vectorial absorption and secretion by the intestinal epithelium. During an infection, the redistribution of apical and basal proteins provides new attachment sites for bacteria. Several bacterial toxins act on the TJ protein functions inducing an epithelial damage. At least four toxins produced by *Clostridium spp.* and a toxin produced by selected *E. coli* strains have been demonstrated to affect intestinal TJ [9, 10].

In this complex scenario, different bacterial species, each with one or more virulence factors, induce diarrhea in a specific mode. This includes the interaction with specific receptor, the release of virulence factors such as adhesion molecules and enterotoxins and a cascade of events occurring within the enterocyte.

Bacterial Diarrhea

Vibrio Cholerae

This pathogen is easily identified in the stools using Gram stain and is responsible for cholera, a potentially fatal diarrheal disease in humans. Although cholera is now rare in developed countries, it remains a major cause of diarrheal morbidity and mortality in several developing countries. Indeed, with the occurrence of calamities, the spreading of cholera

infection in overcrowded refugee camps is a potential significant threat everywhere. The infection is transmitted by the fecal–oral route and is spread through contaminated food and water, the period of incubation ranges from few hours to 5 days. The vast majority of infected subjects remain asymptomatic or experience a mild disease with watery stools, nausea or vomiting, and no significant dehydration. However, cholera may be severe in a number of subjects. Stools are classically described as “rice water” due to the presence of mucus in clear stools. Profuse watery diarrhea and vomiting lead to massive fluid and electrolyte losses that can occur at a rate of 1 l/h, causing a massive dehydration.

Virtually, all cases of non-O1 *Vibrio* infections in the USA are associated with eating raw shellfish and gastroenteritis ranges from a mild illness to profuse, watery diarrhea comparable to that seen in epidemic cholera. Diarrhea, abdominal cramps, and fever are the most common symptoms with nausea, vomiting, and bloody stools occurring less frequently. As with *V. cholerae* O1, the mainstay of therapy for diarrheal disease is oral rehydration [11, 12]. In cases of septicemia (which typically occurs in immunocompromised patients), supportive care and correction of shock are essential interventions associated with antibiotics (tetracycline). *V. cholerae* secretes several toxins, but the most important of these is cholera toxin (CT). CT consists in a single copy of the A subunit and five copies of the B subunit. B subunit binds to plasma membrane, while the A subunit activates adenylate cyclase resulting in elevated cAMP production. The production of cAMP activates the protein kinase A (PKA), which then phosphorylates the regulatory domain of CFTR [5, 13]. In addition to increased Cl⁻ secretion, absorption of Na⁺ is decreased through a cAMP-dependent mechanism in which the activity of both apical sodium transporters, NHE2 and NHE3, is decreased. This leads to an increase in NaCl levels in the intestinal lumen and drives water by osmotic force [14].

In addition to CT, *V. cholerae* produces other toxins that modulate ion secretion and alter barrier function to cause massive diarrhea. The toxins that directly affect ion secretion include the accessory CT (ACE), which stimulates Ca²⁺-dependent Cl⁻ secretion, the non-agglutinable *Vibrio cholerae* heat-stable enterotoxin (NAG-ST), which activates guanylyl cyclase, thus stimulating cGMP production, leading to protein kinase G (PKG)-mediated activation of CFTR and, finally, the *V. cholerae* cytolysin (VCC), which creates anion permeable pores into the cell wall [15].

Salmonella

Salmonella typhi and *Salmonella paratyphi* are Gram-negative, motile bacilli that colonize only humans. Therefore, the infection is acquired through close personal contact or through the ingestion of water or food contaminated with

human feces. Typhoid fever continues to represent a global health problem, with more than 12.5 million cases/year [16]. After an incubation period ranging between 5 and 21 days, these bacteria cause a systemic illness characterized by fever, gastrointestinal symptoms, and occasionally neurological symptoms [17]. Chills, headache, cough, weakness, and muscle pain are frequent prodromes and most symptoms resolve within 4 weeks without antimicrobial treatment. However, *Salmonella* may spread and invade the bloodstream and extraintestinal districts causing a systemic disease defined as enteric fever. In this case, patients relapse with high fever, abdominal pain from inflammation of Peyer’s patches, and intestinal microperforation followed by secondary bacteremia with intestinal agents.

In contrast to *S. typhi*, infections with non-typhoidal salmonellae are increasing in developed countries. Patients at higher risk for infection include those with immunodeficiencies, age younger than 3 months, alterations in intestinal defenses (e.g., on those antacid treatment), impaired reticuloendothelial function (sickle cell and hemolytic anemia), and ingestion of antibiotics to which the organism is resistant. Reservoirs include a wide range of domestic and wild animals, including poultry, swine, cattle, rodents, and reptiles. *Salmonella enteritidis* is the leading reported cause of food–borne diarrheal outbreaks in the USA, with eggs and contaminated raw fruits and vegetables identified as major vehicles [18].

The incubation period is 6–45 h, after which fever, headache, vomiting, abdominal pain, and watery stools (which may contain blood, mucus, and leukocytes) appear, lasting from locus to few days. Severe extraintestinal infections can range from life-threatening sepsis to focal infections in the meninges, bones, and lungs. The microorganism is easily isolated from fresh stools or blood culture.

Clostridium Difficile

Clostridium difficile (CD), a Gram-positive anaerobe that forms spores (hence difficult to clear from hospital setting), is now recognized as the most common agent of nosocomial diarrhea [19]. In the past decade, a dramatic increase in the incidence of *C. difficile* infections (CDI) has been reported worldwide, possibly linked with an excess of antibiotics and other treatment responsible for microflora disruption but also linked with emergence of hypervirulent strains (e.g., NAP1/BI/027) and increased numbers of highly susceptible individuals.

Antibiotic treatment predisposes patients to *C. difficile*-associated disease in specific conditions [20]. The pathogenesis of *C. difficile* infection largely depends on the altered balance of the intestinal microbiota, allowing pathogenic strains of *C. difficile* to infect the intestine [21].

C. difficile causes a broad spectrum of intestinal diseases ranging from mild diarrhea to potentially fatal pseudomembranous colitis (PMC). *C. difficile* is a major agent of antibiotic-associated colitis [22] and produces three toxins, toxin A (TxA), toxin B (TxB), and a binary toxin (cytotoxic distending toxin, CDT) [23]. Both TxA and TxB exert a cytotoxic effect in vitro [24, 25]. In particular, TxA and TxB induce the disaggregation of the actin cytoskeleton, cell rounding, cell death, and loss of intestinal epithelium barrier function leading to increased intestinal permeability and diarrhea [26]. CDT induces a redistribution of microtubules and the formation of long microtubule-based protrusions at the surface of intestinal epithelial cells [9] enhancing adherence and colonization of Clostridia [25, 27].

However, diarrhea is essentially due to a necroinflammatory reaction: *C. difficile* toxins trigger an extensive inflammatory cascade causing a rapidly progressive damage to host tissues resulting in fluid exudation [28]. In parallel, selected cytokines are also able to induce the activation of enteric nerves and induce Cl⁻ secretion. The onset of symptoms may begin from several days after antibiotic therapy is started until 2 months following cessation of treatment. Diarrhea and abdominal cramps are usually the presenting symptoms, followed by fever and chills in severe cases. Mild colitis, with bloody stools and mucus, particularly if they follow antibiotic treatment, should be considered potentially induced by *C. difficile* infection. Microbial culture, latex agglutination, tissue culture assay, and enzyme-linked immunosorbent assay (ELISA) are all used for the diagnosis of *C. difficile* infection.

However, the role of *C. difficile* in infancy and early childhood is still a matter of debate. This is an emerging agent of diarrhea whose role is limited or questionable in children below 36 months of age [29, 30]. It is also a major agent of antibiotic-induced diarrhea and of severe diarrhea in children with underlying chronic conditions such as inflammatory bowel diseases (IBD) [31].

Asymptomatic *C. difficile* colonization is common in infants (ranging from 2 to 75% of healthy children) until 3 years of age and related to delivery mode and feeding [32]. Strains that usually are associated with diarrhea in adults may be detected in asymptomatic infants, and children may be a reservoir of pathogenic strains.

Many healthy children excrete *C. difficile* in their stools during early infancy, and rates as high as 45% have been detected in infants attending day care nurseries and as many as 13% were harboring toxigenic isolates [33].

Independently from the prevalence of carriers and the method to search for *C. difficile*, the incidence of *C. difficile*-related diseases in children has progressively increased in the last years, in parallel with data reported in adults, but with a relative more limited clinical impact. A recent 18-year cohort study reported a 12-fold increase of *C. difficile* infection incidence in children. Overall *C. difficile* infections

increased from 2.6 (1991–1997) to 32.6 per 100,000 persons (2004–2009), with a prevalence of community acquired infection that increased from 2.2 to 23.4 per 100,000 in the same period [34]. However, a role of *C. difficile* as a severe pathogen in children at risk is supported by a solid data: For this reason, one potential alternative to standard therapy is the use of indigenous intestinal microorganisms from a healthy donor to restore the intestinal microbiota of infected patients [35, 36].

In children with chronic diseases such as IBD, *C. difficile* may act as severe opportunistic pathogen and trigger burst of the disease with a potentially severe course.

Shigella

Shigella causes 250 million cases of diarrhea and 650,000 deaths worldwide per year. *Shigella* is a Gram negative, non-lactose fermenting, nonmotile bacillus. *Shigella sonnei* is the main type in industrialized countries, and *S. flexneri* and *S. dysenteriae* predominating in developing countries [37]. The latter is the most dangerous species as it produces Shiga toxin, which can lead to hemolytic uremic syndrome (HUS). Humans are the only natural hosts and transmission occurs by fecal–oral contact. The very low infective load (as few as ten organisms) makes *Shigella* highly contagious. *Shigella* causes a dysenteric diarrhea, and the cellular responses to various steps of the invasion process are the primary cause of inflammation. *Shigella* strains cross the epithelial barrier through the M-cells where they bind to basolateral TLR4 receptor. This causes the production of interleukin (IL)-6, IL-8, and the subsequent release of IL-1 β , which attracts PMN cells [38]. Nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK)-signaling pathways are activated not only in *Shigella*-infected epithelial cells but also in uninfected bystander cells [39], and this cell–cell communication amplifies inflammation. However, *Shigella* extensively disrupts TJs. In polarized cultured T84 epithelial cells, ZO-1, claudin-1, and the phosphorylation status of occludin were all affected by *S. flexneri* [40]. There is a secondary mechanism that promotes diarrhea, triggered by serine protease autotransporters of *Enterobacteriaceae* (SPATES), three enterotoxins that alter transepithelial fluid movement and cytoskeletal structure [41]. After 1–4 days of incubation, shigellosis begins with fever, headache, malaise, anorexia, and occasional vomiting and watery diarrhea with progression to dysentery within hours to days. Unusual extraintestinal manifestations may occur, including HUS in children and thrombotic thrombocytopenic purpura in adults. Most episodes of shigellosis in otherwise healthy individuals resolve within 7 days. *Shigellae* are difficult to grow and are best isolated from fresh stools rapidly inoculated into selective culture plates incubated immediately at 37°C.

Campylobacter jejuni

These organisms are small, spiral-shaped Gram-negative bacilli that live in the intestines of both wild and domestic animals [42]. Common vehicles for human infection are poultry, unpasteurized milk, and contaminated water [43, 44]. After 3–6 days of incubation, symptoms abruptly begin. *Campylobacter* diarrhea may present as a typical gastroenteritis with vomiting and profuse diarrhea or as a colitis with abdominal pain and bloody stools, and abdominal pain that may mimic an appendicitis. Diarrhea usually lasts 4–5 days; the microorganism can be identified only from stool. *Campylobacter* vaccine development has proceeded cautiously, because of concerns about postexposure arthritis or Guillain–Barré syndrome. However, a monovalent, formalin-inactivated, *C. jejuni* whole-cell vaccine with a mucosal adjuvant has entered human trials.

Yersinia

Yersinia enterocolitica and *Y. pseudotuberculosis* are two important human enteropathogens widely distributed in the environment, with swine as the major reservoir. The incubation period is 3–7 days, with food-borne transmission. *Yersinia*'s preference for cool temperatures makes this pathogen more common in Northern Europe, Scandinavia, Canada, the USA, and Japan. *Yersinia* enterocolitis occurs more often in children younger than 5 years [45] and is characterized by fever, vomiting, exudative pharyngitis, cervical adenitis, abdominal pain, and watery diarrhea, which may contain blood [46, 47]. Diarrhea typically lasts for 14–22 days, but fecal excretion may persist for 7 weeks or longer. Abdominal complications include appendicitis, pseudoappendicitis, diffuse ulcerations of the intestine and colon, intestinal perforation, peritonitis, ileocecal intussusception, toxic megacolon, cholangitis, and mesenteric vein thrombosis. Bacteremic spread may result in abscess formation and granulomatous lesions in the liver, spleen, lungs, kidneys, and bone, as well as meningitis and septic arthritis. *Yersinia* infection can also be associated with extraintestinal sequelae including reactive arthritis, uveitis, Reiter's syndrome, and erythema nodosum [48]. *Yersinia* may be isolated from stools or pharyngeal exudates on commonly used selective media, and appears as Gram-negative colonies after 2–14 days of growth at 25–28°C.

E. coli

E. coli is the most abundant facultative anaerobe of the human colonic flora and typically colonizes the gastrointestinal tract within few hours after birth. *E. coli* is a Gram-

negative, lactose-fermenting motile bacillus of the family *Enterobacteriaceae*. Currently, 171 somatic (O) and 56 flagellar (H) antigens are recognized. *E. coli* includes a heterogeneous group of microorganisms capable to exert various possible interactions with the host, ranging from a role of mere harmless presence to that of a highly pathogenic organism [49]. Six distinct categories of *E. coli* are currently recognized as pathogens: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC or EAggEC), and enteroinvasive *E. coli* (EIEC).

ETEC ETEC strains elaborate two classes of enterotoxins, namely the heat-labile and the heat-stable enterotoxins (LT and ST, respectively) [50]. LT, the heat-labile enterotoxin, is the analogue of CT and induces chloride secretion by increasing the intracellular cAMP levels. ST, the heat-stable enterotoxin, is a small peptide that causes an increase of intracellular cGMP to induce chloride secretion and diarrhea [51]. The jejunum is a major target of ST-induced anion secretion that is mediated by CFTR [27]. ST binds to its receptor guanylate cyclase C (GCC) on the apical surface of enterocytes, resulting in the generation of cGMP. This in turn activates a cGMP-dependent kinase (cGKII) leading CFTR phosphorylation [50] and the consequent inhibition of Na⁺ absorption through the apical membrane of jejunal enterocytes [48]. ST does this by acting on both the apical and the basolateral side of intestinal epithelium, providing an example of a molecular mimicry between ST and its endogenous ligand guanylin, an endogenous analogue found both in the intestinal lumen and in the blood that regulates two distinct processes, ion absorption and cell proliferation [52, 53]. Transmission of *E. coli* occurs by ingestion of contaminated food and water, with peaks during the warm, wet season. Like EPEC, ETEC requires a relatively high inoculum and has a short incubation period (14–30 h). The key symptom is watery diarrhea, sometimes with fever, abdominal cramps, and vomiting. In its most severe form, ETEC can cause cholera-like severe diarrhea and dehydration. The illness is generally self-limited, lasting less than 5 days. Infection with ETEC has also been associated with short- and long-term nutritional consequences in infants and children.

EPEC This was the first group of *E. coli* serotype shown to be pathogenic for humans and responsible for devastating outbreaks of nosocomial neonatal diarrhea and infant diarrhea in virtually every corner of the globe. EPEC is characterized by specific serogroups, the most common being *E. coli* O145, O49, and O157. Some of those strains have additional virulence factors. EPEC strains are peculiar in their ability to induce a characteristic attaching and effacing lesion in the small-intestinal enterocytes and by their inability to produce Shiga toxins.

However, EPEC encode a T3SS and produces a characteristic attaching and effacing (A/E) lesion which is characterized by effacement of microvilli on the epithelial surface at the site of bacterial attachment. This typical lesion is responsible for the loss of absorptive surface and osmotic diarrhea [54]. Intestinal pathogens can disrupt the barrier by directly altering the distribution or phosphorylation status of TJ proteins. EPEC strains alter the phosphorylation status and distribution of TJ proteins, occludin and claudin-1, thereby disrupting the epithelial barrier function [10]. This increases paracellular permeability driving ion and water into the lumen upon electrochemical gradients ultimately causing diarrhea. The link between barrier disruption and diarrhea derives from studies involving the pro-inflammatory cytokine, tumor necrosis factor- α (TNF α) [55, 56]. These observations suggest that EPEC-induced diarrhea is a multifactorial process with alterations of electrolyte, solute, and water transport. EPEC causes a self-limited watery diarrhea with a short incubation period (6–48 h). This may be associated with fever, abdominal cramps, and vomiting, and EPEC is a leading cause of persistent diarrhea (lasting 14 days) in children in developing countries [57].

The microbiological diagnosis of EPEC-induced disease is performed with analytic methodologies different from those used by the standard microbiology laboratory, the most relevant being: (a) serotyping, (b) adherence assay, (c) FAS test, and (d) the specific detection of virulence-involved genes (bfpA and eae genes) using molecular biology techniques [58].

However, the cause–effect relationship between EPEC in the stools and diarrhea is hampered by the high number of healthy carriers.

EHEC It is another major intestinal pathogen that is a subset of Shiga toxin-producing *E. coli* (STEC). *E. coli* O157:H7 is the prototype of EHEC serotype [59].

EHEC adheres to epithelial cells, expresses a T3SS and causes A/E lesions much like EPEC. Unlike EPEC, infection with EHEC may cause severe symptoms including bloody diarrhea and life-threatening HUS. These symptoms are due to the production of Shiga toxin which elicits luminal fluid accumulation in the intestine [60, 61]. The predominant transmission is through the ingestion of contaminated food. Crampy abdominal pain and non-bloody diarrhea are the first symptoms, sometimes associated with vomiting. Diarrhea always becomes bloody and abdominal pain worsens, lasting 1–22 days. Fever is usually absent or low grade. In outbreaks, approximately 25% of patients are hospitalized, 5–10% develop HUS, and 1% may have a poor outcome. The most dangerous complication of EHEC infection is HUS. This is usually diagnosed 2–14 days after the onset of diarrhea. Risk factors include young age, bloody diarrhea, fever, an elevated leukocyte count, and treatment with antimotility agents [62]. The most widely accepted indica-

tion for seeking *E. coli* O157:H7 infection is a patient with bloody diarrhea, in whom an accurate diagnosis may avoid unnecessary surgery.

DAEC DAEC has an age target ranging from 48 to 60 months and shows a seasonal pattern similar to that of ETEC, occurring more frequently in the warm season. The gastrointestinal symptoms include self-limiting watery diarrhea rarely associated with vomiting and abdominal pain. The diagnosis is mainly based on the DNA probe technique and on the pattern of adherence of the microorganism to HEP-2 cells. Given the technical problems of both assays, their use is limited to epidemiological surveys rather than diagnosis.

EAggEC EAggEC are diarrheagenic germs with the ability of adhere to HEP-2 cells and the intestinal mucosa. They have been especially associated with cases of persistent (i.e., lasting ≥ 14 days) [63, 64] diarrhea both in the developed and developing world [65].

Typical clinical features are a watery, mucoid, secretory diarrheal illness with low-grade fever, and little or no vomiting. However, bloody stools have been reported. Infection of EAggEC is detected by the isolation of *E. coli* from the stools of patients and the demonstration of the aggregative pattern in the HEP-2 assay. Establishing a cause–effect relationship is hampered by the high rate of asymptomatic colonization; if no other organism is implicated in the patients' illness and EAggEC is isolated, the germ should be considered as a potential cause of diarrhea. A DNA-fragment probe has proven highly specific in the detection of EAggEC strains. Acute diarrhea is apparently self-limiting; however, more persistent cases may benefit from antibiotic and/or nutritional therapy, after susceptibility test [66].

EIEC Invasive *E. coli* strains are genetically, biochemically, and clinically nearly identical to *Shigella* [67]. They also show a similar epidemiological pattern and are endemic in developing countries. The role of EIEC in industrialized countries is limited to rare food-borne outbreaks. EIEC can rarely produce dysentery.

Antimicrobial Therapy

Antimicrobial therapy should not be given to the vast majority of otherwise healthy children with acute gastroenteritis. Acute gastroenteritis in a child without significant underlying disease is usually self-limited regardless of the etiology, which is seldom known at the onset of symptoms. Clinical recovery generally occurs within a few days without specific antimicrobial therapy, and the causative organism is cleared in a relatively short time, usually within a few days or weeks. Complications are uncommon.

Antimicrobial Therapy of Bacterial Gastroenteritis

Antibiotic therapy for acute bacterial gastroenteritis is not needed routinely but could be considered for specific pathogens or in defined clinical settings, including clinical condition and risk factors [68, 69].

Pathogen-Based Approach

Shigella Gastroenteritis Antibiotic therapy is recommended for culture-proven or suspected *Shigella* gastroenteritis. The first-line treatment for shigellosis is azithromycin for 5 days. A meta-analysis of 16 studies, which included 1748 children and adults with *Shigella* dysentery, concluded that appropriate antibiotic therapy shortened the duration of the disease. Several well-designed controlled studies have shown that appropriate antibiotic treatment of *Shigella* gastroenteritis significantly reduces the duration of fever, diarrhea, and fecal excretion of the pathogen, and thus infectivity, which is very important in children attending day-care centers [70], those admitted in institutions and hospitals. Antibiotic treatment may also reduce complications including the risk of HUS after *S. dysenteriae* infection.

WHO [71] recommends that all episodes of *Shigella* infection be treated with ciprofloxacin or one of the 3-s line antibiotics (pivmecillinam, azithromycin, or ceftriaxone). The major problem, however, is the increasing worldwide resistance of *Shigella* to antibiotics that is also being observed in Europe [72, 73]. Therefore, *Shigella* isolates should be tested for susceptibility, and local resistance pattern be closely monitored [74]. A systematic review of data from 1990 to 2009 identified 8 studies in children up to 16 years with shigellosis reporting clinical failure 3 days after treatment. In addition, four studies evaluated bacteriologic failure and five assessed bacteriologic relapse. Clinical failure rate was 0.1% and bacteriologic relapse was 0%. Based on these figures, which however derive from low-income countries, antibiotic therapy is consistently effective and strongly recommended in all children with shigellosis. It should be noted, however, that this finding has not been demonstrated in outpatients. Because of the high worldwide resistance, trimethoprim-sulfamethoxazole (TMP-SMX) and ampicillin are recommended only if the strain isolated is susceptible, or if current local microbiologic data suggest susceptibility. In Europe and the USA, resistance to ceftriaxone, azithromycin, and ciprofloxacin has been reported, but is uncommon. The first-line oral empiric treatment recommended for *Shigella* gastroenteritis is azithromycin for 5 days [72, 73]. Alternatively, nalidixic acid or cefixime can be administered for 5 days. When *Shigella* isolates are susceptible to (TMP-SMX) and/or ampicillin (i.e., in an outbreak setting), these agents are recommended as first-line treatment. Oral fluoroquinolones can be used in children younger than 17 years

when no other alternative is feasible. The recommended first-line parenteral treatment is ceftriaxone for 5 days. Two doses of ceftriaxone can be given to patients without underlying immune deficiency or bacteremia who are fever-free after 2 days of ceftriaxone treatment [75].

Salmonella Gastroenteritis Antibiotic therapy is not effective and does not prevent complications. Rather, it is associated with a prolonged fecal excretion of *Salmonella*. Therefore antibiotics should not be used in an otherwise healthy child with *Salmonella* gastroenteritis. Antibiotics are suggested in high-risk children to prevent the risk of bacteremia and extraintestinal infections. These include neonates and young infants (<6 months) and children with underlying immune deficiency, anatomical or functional asplenia, corticosteroid or immunosuppressive therapy, IBD, or achlorhydria (weak recommendation, low-quality evidence). A Cochrane systematic review showed that antibiotic therapy of *Salmonella* gastroenteritis does not significantly affect the duration of fever or diarrhea in otherwise healthy children or adults compared to placebo or no treatment [76]. Moreover, antibiotics were associated with a significant increase of carriage of *Salmonella*, although other adverse events were not reported. As secondary *Salmonella* bacteremia—with potential extra-intestinal focal infections—occurs more often in children with certain underlying conditions, and in neonates or young infants; antibiotic therapy with TMP-SMX, ampicillin (to which 10–20% of isolates in the USA are resistant), cefotaxime, ceftriaxone, or chloramphenicol, is suggested in these children to reduce the risk of bacteremia.

Campylobacter Gastroenteritis Antibiotic therapy for *Campylobacter* gastroenteritis is recommended mainly for the dysenteric form and to reduce transmission in day-care centers and institutions. It reduces symptoms if instituted in the early stage of the disease (within 3 days after onset). The first-line drug is azithromycin, but the choice should be based on local resistance pattern. A meta-analysis of 11 double-blind, placebo-controlled trials showed that antibiotic treatment of gastroenteritis caused by *Campylobacter* spp. reduces the duration of intestinal symptoms by 1.3 days. The effect was more pronounced if treatment was started within 3 days of illness onset and in children with *Campylobacter*-induced dysentery. Antibiotic treatment significantly reduces the duration of fecal excretion of *Campylobacter* spp. and thus its infectivity. Azithromycin is the drug of choice in most locations, although local resistance patterns should be closely monitored.

Diarrheagenic E. coli Antibiotic should not be routinely given for *E. coli*. The treatment is nonspecific and administration of antibiotic may have adverse effect. Antibiotic therapy for Shiga toxin-producing *E. coli* is not recom-

mended. Antibiotic therapy for enterotoxigenic *E. coli* is recommended [77]. Antibiotic treatment of diarrhea induced by Shiga toxin-producing *E. coli* (STEC), also called EHEC, does not significantly affect the clinical course or duration of fecal excretion of the pathogen. Antibiotic treatment is not routinely indicated of gastroenteritis caused by enterotoxigenic *E. coli* or by enteropathogenic *E. coli*.

Most diarrheal illnesses due to ETEC are self-limited and do not require specific antimicrobial therapy. Empiric therapy is reserved for severe cases despite rehydration and supportive measures. The following antibiotics can be considered based on resistance pattern: doxycycline, TMP-SMX, ciprofloxacin, quinolones, and furazolidone. A large proportion of ETEC is now resistant at TMP-SMX, used especially for children, so an alternative regimen is furazolidone. Prevention of ETEC infection is based on avoiding contaminated vehicles. Although few data exist to guide antibiotic therapy of EPEC diarrhea, administration of appropriate antibiotics seems to diminish morbidity and mortality [77]. A 3-day course of oral, nonabsorbable antibiotics such as colistin or gentamicin (if available) has been shown to be effective [78]. Also Rifaximin [79], a broad-spectrum, nonabsorbed antimicrobial agent, can be considered in children > 12 years and in adults for nonfebrile watery diarrhea presumably caused by enterotoxigenic or EAaggEC gastroenteritis.

Antibiotics are not routinely needed in EPEC-induced diarrhea, otherwise EIEC treatment is identical to shigellosis, but antibiotics are rarely needed.

C. difficile In many instances, *C. difficile*-induced antibiotic diarrhea is self-limiting, and the patient may respond simply to the withdrawal of the offending antibiotic. In more severe forms, particularly if complicated by PMC, antibiotic treatment with either oral vancomycin (5–10 mg/kg, maximum 500 mg, given every 6 h for 7 days) or metronidazole (5–10 mg/kg, maximum 500 mg, given every 8 h for 7 days) is recommended, although the rate of relapse is very high [80, 81].

The risk of treatment failure and of recurrence parallels the number of *C. difficile* infections episodes, ranging between 15 and 30% in the first infection and reaching 40 and 65% in patients experiencing a second or third episode, respectively [82]. Failure is much lower or not existent with vancomycin as first treatment.

The increasing frequency of *C. difficile* infections recurrence and the relative challenging treatment, led to the development of new therapeutic strategies including different antibiotic approaches (e.g., Fidaxomicin or Vancomycin tapering), active and passive immunotherapy (e.g., specific monoclonal antibodies against *C. difficile* toxins), and microflora restoration through the administration of probiotics or fecal donor microbiota transplantation. However, trials with new therapeutic approaches have been limited in chil-

dren. The use of monoclonal antibodies to target toxins is being considered for treatment of *C. difficile* infections and its recurrence. The neutralization of toxin activity may stop *C. difficile* infections symptoms and prevent recurrences and showed good results in adults with recurrent *C. difficile* infections [83].

This approach, that showed promising results in adults [84], has not yet been tested in children and further studies are required to establish its role in *C. difficile* infections therapy and its cost effectiveness.

The efficacy of probiotics in severe and recurrent *C. difficile* infections cases are conflicting [85], the use of selected probiotics, particularly *Lactobacillus GG* and *Saccharomyces boulardii*, has been associated with a significant eradication of the pathogen and a decrease in the recurrence of the infection [86, 87].

A recent approach for severe or recurrent *C. difficile* diarrhea in high-risk children consists in the instillation of healthy donor feces into the *C. difficile*-infected gastrointestinal tract. This technique, known as fecal microbiota transplantation (FMT), aims at restoring the normal composition and the wide diversity of gut microbiota. Current evidence (although weak) demonstrates consistent and excellent efficacy in clinical outcomes in adults. However, many questions should be answered before it may be recommended including the long-term effects (with the potential association with autoimmune diseases) [88].

Other Causes of Bacterial Gastroenteritis

Antibiotic therapy is recommended for *V. cholerae* gastroenteritis since it reduces the duration of diarrhea by about 50% and bacterial shedding by about 1 day. WHO recommends administration for 3–5 days of furazolidone (1.25 mg/kg four times per day), TMH (5 mg/kg twice per day), SMX (25 mg/kg twice per day), and erythromycin (10 mg/kg three times per day) to children less than 8 years and of tetracycline to older children.

A randomized, controlled study demonstrated that a single dose of 20 mg/kg of azithromycin is clinically and microbiologically more effective than ciprofloxacin. Azithromycin is the drug of choice for children younger than 8 years. Alternative treatment for older children is doxycycline. TMP-SMX can be used for susceptible strains. Both killed whole-cell and live-attenuated cholera vaccines have been proposed as a preventive intervention for cholera; a large double-blind field trial of the killed vaccine showed 85% efficacy for a period of 4–6 months, dropping to 50% over 3 years of follow-up. The introduction of oral rehydration solutions (ORS) [89] dramatically decreased the mortality from this illness.

Limited data are available regarding the efficacy of antibiotics for gastroenteritis caused by *Yersinia* spp., and an-

Table 15.1 Anti-infective therapy and vaccines available

Anti-infective therapy				Vaccines available
Anti-infective therapy	Anti-infective therapy should not be given to the vast majority of otherwise healthy children with acute gastroenteritis	Strong recommendation, low quality of evidence	Va, D	
Antimicrobial therapy of bacterial gastroenteritis	Antibiotic therapy for acute bacterial gastroenteritis is not needed routinely but only for specific pathogens or in defined clinical settings	Strong recommendation, low quality of evidence	Va, D	
<i>Shigella</i> gastroenteritis	Antibiotic therapy is recommended for culture proven or suspected <i>Shigella</i> gastroenteritis	Strong recommendation, moderate quality of evidence	I, B	NO
<i>Salmonella</i> gastroenteritis	Antibiotics should not be used in an otherwise healthy child with <i>Salmonella</i> gastroenteritis	Strong recommendation, moderate quality of evidence	I, A	YES Attenuated vaccine for typhoid fever, Ty21a, administered as a liquid suspension, protected both young and older children; three new generation-attenuated vaccines, genetically engineered, are undergoing extensive phase II trials
	Antibiotics are suggested in high-risk children (neonates and young infants (<6 months) and children with underlying immune deficiency) to reduce the risk of bacteremia and extra-intestinal infections	Strong recommendation, low quality of evidence	Vb, D	
		Weak recommendation, low quality of evidence	Vb, D	
<i>Campylobacter</i> gastroenteritis	Antibiotic therapy for <i>Campylobacter</i> gastroenteritis is recommended mainly for the dysenteric form and to reduce transmission in daycare centers and institutions. It reduces symptoms if instituted in the early stage of the disease (within 3 days after onset)	Strong recommendation, moderate quality of evidence	I, A	NO
Diarrheagenic <i>Escherichia coli</i>	Antibiotics are usually not indicated, but it depends on the specific cause and setting	Weak recommendation, low quality of evidence	Va, D	YES, but only for EHEC: parenteral toxoids and live oral carrier strains elaborating the B subunit of Shiga toxin; vaccines expressing the adhesin intimin, designed to prevent intestinal colonization, and a parenteral O157 polysaccharide protein conjugate. ETEC: oral vaccines including killed whole cell, toxoids, purified fimbriae, living attenuated strains, and live carrier strains elaborating ETEC antigens
Empiric antibiotic Therapy in sporadic cases of AGE	The choice of the antimicrobial agent depends on the local prevalence of the causative pathogens and the local resistance patterns	Strong recommendation, moderate quality of evidence	Va, B	
	In children with watery diarrhea, antibiotic therapy is not recommended unless the patient has recently traveled or may have been exposed to cholera	Strong recommendation, moderate quality of evidence	Vb, D	
	In bloody diarrhea with low or no fever, antibiotics are not recommended unless epidemiology suggests shigellosis	Strong recommendation, low quality of evidence	Vb, D	
	Parenteral rather than oral antibiotic therapy is recommended for: Patients unable to take oral medications Patients with underlying immune deficiency who have AGE with fever Severe toxemia, suspected or confirmed bacteremia Neonates and young infants (<3 months) with fever Sepsis workup and antibiotics should be considered according to local protocols	Strong recommendation, low quality of evidence	Va, D	

EHEC enterohemorrhagic *E. coli*

tibiotics are recommended for bacteremia, extraintestinal infections, and immunocompromised hosts. Production of beta-lactamases generally makes *Yersinia* resistant to cephalosporins and aztreonam, and imipenem are also ineffective. Treatment should generally last 2–6 weeks, with an initial intravenous administration (third-generation cephalosporin often in combination with aminoglycosides), followed by an oral one to which the clinical isolate is sensitive.

Antibiotic therapy is usually not needed for gastroenteritis caused by non-cholera *Vibrio* spp., *Aeromonas* spp., or *Plesiomonas shigelloides*.

Antibiotic-Associated Diarrhea

Antibiotic therapy is generally not needed for antibiotic-associated diarrhea, but should be considered in moderate to severe forms. Antibiotic-associated diarrhea can be defined as a change in normal stool. *C. difficile* is the most frequent agent of this syndrome [79]. Symptoms may range from a mild form of liquid diarrhea to a severe form of PMC with abdominal pain, local, and systemic inflammation and a typical history of diarrhea that is chronologically related with antibiotic administration. It may start from locus to 2 months after starting antibiotics.

Empiric Antibiotic Therapy in Sporadic Cases of Acute gastroenteritis (AGE)

The cause of sporadic AGE is usually not known at presentation and generally it will not be identified. The classification of these cases into invasive (or inflammatory) and watery (or noninvasive) may help deciding whether or not to start empiric antibiotics [90, 91]. Invasive (inflammatory) enteritis is defined as acute onset of bloody/mucous diarrhea (or fecal PMNs leukocytes when the examination is available) with high fever. The common causes are *Shigella* spp., *Campylobacter* spp., and *Salmonella enterica*. Antibiotics should be considered in hospitalized children and children attending daycare centers to reduce transmission of *Shigella* and *Campylobacter*. The choice of the antimicrobial agent depends on the local prevalence of the three pathogens (*Shigella* spp., *Campylobacter* spp., and *Salmonella enterica*) and the resistance patterns.

In children with watery diarrhea, antibiotic therapy is not recommended unless the patient has recently traveled or may have been exposed to cholera [89, 92]. Bloody diarrhea with low or no fever is typical of STEC (EHEC), but can be caused by mild shigellosis associated with salmonellosis. Antibiotics are not recommended unless epidemiology suggests shigellosis. Parenteral rather than oral antibiotic therapy is recommended for [90]:

1. Patients unable to take oral medications (vomiting, stupor, etc)
 2. Patients with underlying immune deficiency who have AGE with fever
 3. Severe toxemia, suspected or confirmed bacteremia
 4. Neonates and young infants (<3 months) with fever
- Sepsis workup and antibiotics should be considered according to local protocols.

Antimicrobial Therapy of Systemic Infections due to Enteric Pathogens or Involvement of Extraintestinal Organs

Antibiotic therapy is recommended for the rare but potentially severe extraintestinal infections caused bacterial enteric pathogens.

Occasionally enteric bacterial pathogens can spread and cause extraintestinal infections, including bacteremia or focal infections. These infections should be treated with antibiotics, usually through the parenteral route [75].

Table 15.1 shows anti-infective therapies and vaccines available.

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