or absence of air, and microaerophilic bacteria can grow in 10% CO_2 in air or under aerobic or anaerobic conditions. As opposed to several anaerobic species inhabiting human bodily surfaces, which can survive only under strict anaerobic conditions (<0.5% oxygen), anaerobes that commonly cause human infections (*Bacteroides fragilis, Prevotella melaninogenica*, and *Fusobacterium nucleatum*) are generally aerotolerant (i.e., they tolerate 2%–8% oxygen) and can survive for sustained periods, but cannot replicate, in an oxygenated atmosphere.

ANAEROBES IN THE NORMAL HUMAN MICROBIOTA

Anaerobic bacteria are the predominant forms of life in the human body. Several hundred species of anaerobic organisms have been identified in the human microbiota[1,2]; however, many of them cannot be characterized by cultivation in vitro, which has been the cornerstone of microbiology since the 19th century. In an analysis of 13,555 prokaryotic ribosomal RNA (rRNA) gene sequences from the colon, most bacteria identified were considered uncultivated and novel microorganisms.[3] New technologies based on DNA analyses expanded researchers' knowledge, and two main projects, the Human Microbiome Project, carried out by the National Institutes of Health,[4] and the European Metahit, aim to characterize the normal microbiota in healthy individuals.

Increasing evidence indicates that human microbiota composition is influenced by diet, geography, and environmental exposure.[5] Anaerobes are dominant in mucosal surfaces, such as the oral cavity and the gastrointestinal (GI) and female genital tracts. These sites account for 99% to 99.9% of the culturable microbiota. The microbial species and concentrations vary at different sites (Table 242.1). It is interesting that anaerobes also inhabit areas of the body that are exposed to air: skin, nose, mouth, and throat. It has been hypothesized that the ability of anaerobes to withstand oxygen at these sites is due in part to the presence of aerobes and facultative organisms that consume oxygen and reduce the oxidation-reduction potential. In addition, anaerobes are believed to reside in the portions of these sites that are relatively well protected from oxygen, such as gingival crevices.

Anaerobes normally abound in the oral microbiota, with concentrations ranging from 10^2/mL in saliva (*Veillonella parvula* being predominant) to 10^{12}/mL in gingival scrapings. The ratio of anaerobic to aerobic bacteria ranges from 1:1 on teeth to 1000:1 in the gingival crevices. The indigenous oral anaerobic microbiota primarily comprises *Prevotella* and *Porphyromonas* spp., with *Fusobacterium* and *Bacteroides* (non–*B. fragilis* group; see later) present in lower numbers. Until recently the lower respiratory tract was considered sterile below the larynx; however, more recent studies using culture-independent techniques have documented the presence of a respiratory tract microbiota that extends from the nasal passages to the alveoli.[6]

Low numbers of anaerobic bacteria are present in the normally acidic conditions of the stomach and upper intestine. In people with decreased gastric acidity, the microbiota of the stomach resembles that of the oral cavity. The upper intestine contains relatively few organisms until the distal ileum, where the microbiota begins to resemble that of the colon. A stagnant proximal, small intestinal segment caused by stricture, obstruction, diverticulum, or blind loop results in colonic concentrations of bacteria with a predominance of anaerobes. In the colon there are up to 10^{12} organisms per gram of stool, with anaerobes outnumbering aerobes by approximately 1000:1 and accounting for 99.9% of the total bacterial burden. The predominant culturable anaerobes are *Bacteroides* spp. (principally members of the *B. fragilis* group, including *B. fragilis, B. thetaiotaomicron, B. ovatus, B. vulgatus, B. uniformis,* and *Parabacteroides distasonis*) and *Clostridium, Peptostreptococcus,* and *Fusobacterium* spp. However, many of the bacteria in the human colon cannot be cultivated by current laboratory methods.

The normal female genital tract is colonized by 10^7 to 10^9 bacteria per mL, with an anaerobic-to-aerobic ratio of 1:1 to 10:1. The predominant anaerobic species are *Prevotella, Bacteroides, Fusobacterium, Clostridium*, and the anaerobic *Lactobacillus* spp. *Bacteroides* spp. are found in the genital tract of approximately 50% of women, with *B. fragilis* making up less than 15% of this microbial population.[7] The skin microbiota contains anaerobes as well, the predominant species being *Cutibacterium* (formerly *Propionibacterium*) *acnes* and, to a lesser extent, other species of *Cutibacterium* and *Peptostreptococcus.*

TABLE 242.1 Comparison of the Anaerobic Human Microbiota at Mucosal Surfaces

ANATOMIC SITE	SAMPLED SITE	TOTAL BACTERIAL NUMBERS (per g/mL)	ANAEROBE: AEROBE RATIO
Upper airways	Nasal washings	10^3–10^4	3–5:1
	Saliva	10^8–10^9	1:1
	Tooth surface	10^{10}–10^{11}	1:1
	Gingival crevices	10^{11}–10^{12}	10^3:1
Gastrointestinal tract	Stomach	0–10^5	1:1
	Jejunum/ileum	10^4–10^7	1:1
	Terminal ileum and colon	10^{11}–10^{12}	10^3:1
Female genital tract (endocervix and vagina)		10^7–10^9	1–10:1

THE MICROBIOME IN HEALTH AND DISEASE

Commensal bacteria in general and commensal anaerobes in particular have been implicated as crucial mediators of several physiologic, metabolic, and immunologic functions in the mammalian host (see also Chapter 2). The occupation of distinct ecologic niches within the intestinal environment that would otherwise be filled with potentially pathogenic organisms is among the most important roles that anaerobes serve as components of the normal colonic microbiota. In what is termed *colonization resistance,*[8] the presence of anaerobes effectively interferes with colonization by potentially pathogenic bacterial species through the depletion of oxygen and nutrients, the production of enzymes and toxic end products, and the modulation of the host's intestinal innate immune response. For example, *Bacteroides thetaiotaomicron* stimulates Paneth cells to produce RegIIIγ, a bactericidal lectin that can result in killing of gram-positive bacteria.[9] The normal colonic microbiota plays an important role in protection against *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea or colitis, a toxin-mediated, potentially life-threatening disease that results when *C. difficile* spores in the colon transform to vegetative forms with toxin production because of antibiotic elimination of critical components of the competing colonic microbiota and a consequent decrease in microbiota diversity. Changes in the microbiota are accompanied by changes in the metabolome that support *C. difficile* germination and growth (see Chapter 243).[10]

A large body of evidence, including randomized controlled trials, systematic reviews, and meta-analyses, provides clear evidence that fecal microbiota transplantation is a highly effective treatment against recurrent *C. difficile* infection.[11,12] Beyond the treatment of *C. difficile* infection, fecal microbiota transplantation has been investigated in other disorders associated with alteration of the gut microbiota, in particular, ulcerative colitis and metabolic syndrome, but at present there are no definitive conclusions.

The anaerobic component of the intestinal microbiota is also responsible for the production of secreted products that are helpful in human health. The production of vitamin K by anaerobes in the intestine is beneficial to the host, and the production of bile by these organisms is useful in fat absorption and cholesterol regulation. The gut microbiota is integral to the host's digestion and nutrition. Microbiota components can generate nutrients from substrates that are otherwise indigestible by the host. For example, carbohydrate fermentation by *Bacteroides* and other intestinal bacteria results in the production of volatile fatty acids that are reabsorbed and used by the host as an energy source.[13] The capacity of gut microbial digestion of xyloglucans, commonly found in dietary vegetables, has been mapped to a single locus in a certain species of *Bacteroides*.[14]

The microbiota contributes to the development and homeostasis of the immune system. The anaerobic intestinal microbiota influences the development of an intact mucosa and of mucosa-associated lymphoid tissue. Germ-free animals exhibit reductions in vascularity, digestive enzyme activity, and muscle wall thickness as well as undeveloped gut-associated lymphoid tissue. Colonization of these mice with a single species, *B. thetaiotaomicron,* affects the expression of various host genes that influence nutrient uptake, metabolism, angiogenesis, mucosal barrier function, and development of the enteric nervous system.[15] Through its symbiosis factor polysaccharide A (PSA), *B. fragilis* influences the development and function of the immune system[16] and protects mice against colitis in a model of inflammatory bowel disease.[17] In addition, PSA can confer protection both prophylactically and therapeutically, restrain inflammatory processes, and ameliorate disease in an extraintestinal site in a mouse model of multiple sclerosis.[18,19]

Although the gut microbiota confers many benefits (as discussed previously), its dysregulation, termed *dysbiosis*, may play a role in the pathogenesis of diseases characterized by inflammation and aberrant immune responses, such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, asthma, and type 1 diabetes.[20,21] Furthermore, the gut microbiota has been associated with obesity, metabolic syndrome,[22,23] cardiovascular disease,[24] and even mental illness.[25]

ETIOLOGY AND MICROBIOLOGY OF ANAEROBIC CLINICAL INFECTIONS

Despite the number of anaerobic species represented in the normal human microbiota, relatively few are involved in human infections. Infections involving anaerobes are often polymicrobial and usually result from the disruption of mucosal surfaces by surgery, trauma, tumors, or ischemia and the subsequent infiltration of resident microbiota. Cecal contents are the source of microorganisms in the case of intraabdominal infections after disruption of intestinal continuity and contamination of the peritoneal cavity. Infections of the head and neck are caused by the commensal microbiota of the mouth. After contamination of previously sterile sites by the mucosal microbiota, the relatively few anaerobic bacteria that survive in the infected site are those that have resisted changes in oxidation-reduction potential and host defense mechanisms.

Table 242.2 shows the gram-negative and gram-positive anaerobes most commonly isolated from clinical specimens.

TABLE 242.2 Anaerobes Commonly Found in Human Infections

Gram-Negative Bacilli

Bacteroides fragilis group: *B. fragilis, B. thetaiotaomicron, Parabacteroides distasonis, B. ovatus, B. vulgatus*
Porphyromonas spp.: *P. asaccharolytica, P. gingivalis*
Prevotella spp.:
Pigmented: *P. intermedia, P. melaninogenica, P. corporis, P. denticola, P. loescheii, P. nigrescens*
Nonpigmented: *P. bivia, P. disiens, P. oralis*
Fusobacterium spp.: *F. nucleatum, F. necrophorum, F. varium*
Bilophila spp.: *B. wadsworthia*
Sutterella spp.

Gram-Positive Cocci

Peptostreptococcus spp.: *P. magnus, P. asaccharolyticus, P. anaerobius, P. prevotii, Parvimonas micra* (formerly *Peptostreptococcus micros*)
Others: *Coprococcus, Peptococcus, Ruminococcus*
Microaerophilic streptococci: (not true anaerobes)

Gram-Positive Non–Spore-Forming Bacilli

Cutibacterium (formerly *Propionibacterium*) spp.: *C. acnes*
Bifidobacterium spp.: *B. dentium*
Lactobacillus spp.
Eubacterium spp.: *E. lentum*
Actinomyces spp.: *A. israelii, A. naeslundii, A. odontolyticus, A. viscosus*

Gram-Positive Spore-Forming Bacilli

Clostridium spp.: *C. perfringens, C. difficile, C. sporogenes, C. sordellii, C. septicum, C. tertium, C. ramosum, C. novyi, C. histolyticum, C. bifermentans, C. innocuum, C. tetani, C. botulinum*

Anaerobic Gram-Negative Bacilli

Among the anaerobic gram-negative bacilli, the *B. fragilis* group is most commonly isolated from human infections.[26] Of this group, *B. fragilis* is the species most often isolated from clinical infections, particularly those emanating from the lower intestine. Other members of this group frequently recovered include *B. thetaiotamicron, B. distasonis, B. ovatus, and B. vulgatus*. These species are all part of the normal GI microbiota and predominate in intraabdominal infections and other conditions that originate from the gut microbiota, such as decubitus ulcer. In the oral cavity the pigmented anaerobes, mainly *Prevotella (P. melaninogenica, P. intermedia), Porphyromonas (P. asaccharolytica),* and nonpigmented *Prevotella (P. oralis, P. oris),* are recognized as species with higher pathogenic potential. They are the predominant anaerobes isolated from respiratory infections and their complications, such as aspiration pneumonia, lung abscess, chronic otitis media, and chronic sinusitis. These species are also isolated from intracranial infections arising from the oral cavity, such as brain abscess.

The fusobacteria *F. nucleatum, F. necrophorum,* and *F. varium,* which normally reside in the oral cavity and the intestinal tract, are often isolated from sites of necrotizing pneumonia and abscesses, including brain abscess. *Prevotella bivia* and *Prevotella disiens* colonize the vagina and are the organisms most frequently isolated from infections arising at this site.

Anaerobic Gram-Positive Cocci

Anaerobic gram-positive cocci are part of the oral, upper respiratory tract, intestinal tract, and skin microbiota and are isolated from anaerobic infections arising from these sites, including chronic sinusitis, mastoiditis, aspiration pneumonia, lung abscess, and necrotizing soft tissue infections (see Chapter 248).

Anaerobic Gram-Positive Non–Spore-Forming Rods

These organisms are part of the microbiota of the gingival crevices, GI tract, vagina, and skin. They include *Cutibacterium* (formerly *Propionibacterium*), *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, *Arcanobacterium*, *Actinomyces*, *Atopobium*, *Mobiluncus*, and *Pseudoramibacter*. The organisms can be isolated from intracranial abscesses, aspiration pneumonia, and peritonitis. *Cutibacterium* spp., of which *C. acnes* is commonest, are ordinarily nonpathogens but can cause infections in implanted prostheses and central nervous system (CNS) shunts and drains.

Anaerobic Spore-Forming Bacilli

Clostridia are the main pathogens among this group. These organisms are commonly isolated from wounds, abscesses, and blood. The most common isolates from clinical infections are *C. perfringens*, *C. septicum*, *C. ramnosum*, *C. novyi*, *C. sordellii*, *C. histolyticum*, *C. fallax*, *C. bifermentans*, and *C. innocuum*.[27] *C. difficile,* which can cause severe intraluminal infections, usually is not invasive.

CLINICAL SYNDROMES CAUSED BY ANAEROBES

Anaerobes are remarkable in their ability to cause a variety of infections at a number of different anatomic sites. Table 242.3 summarizes the types of infections caused by these organisms. Because anaerobes colonize sites that are home to aerobes and also facultative organisms, many infections from which anaerobes are isolated also involve these other bacteria. Fig. 242.1 shows a Gram-stained specimen from a site of mixed infection in a patient with Meleney gangrene, a form of cellulitis involving *Staphylococcus aureus* and anaerobic streptococci.

Certain infections are likely to involve anaerobes as important pathogens, and the presence of these organisms should be assumed. Such infections include oral and dental infections; brain abscess; human and animal bites; aspiration pneumonia and lung abscess; peritonitis after a perforated viscus; infections of the female genital tract (e.g., endometritis, amnionitis, septic abortion, and tubo-ovarian abscess); infections after surgeries involving the GI, oral, or female genital tract; and necrotizing infections of soft tissues and muscles.

The hallmark of infection caused by anaerobic bacteria (mostly gram-negative bacilli) is abscess formation. Typically, abscesses form

TABLE 242.3 Infections Commonly Caused by Anaerobes

Mouth, Head, and Neck
Chronic sinusitis
Dental infection and dental abscess
Periodontitis, periodontal abscess
Gingivitis
Perimandibular infection
Thoracic Cavity
Aspiration pneumonitis
Necrotizing pneumonia
Empyema
Lung abscess
Abdominal Cavity
Peritonitis
Appendicitis
Biliary tract infection
Wound infection
Liver abscess
Intraabdominal abscess
Female Genital Tract
Vulvar and Bartholin gland abscesses
Pelvic inflammatory disease and adnexal abscess
Pelvic abscess
Postsurgery and postpartum infection
Septic pelvic thrombophlebitis
Endometritis, amnionitis, septic abortion
Intrauterine device–associated infection
Bacterial vaginosis
Central Nervous System
Brain abscess
Subdural empyema
Epidural abscess
Skin and Soft Tissue
Diabetic foot ulcer
Decubitus ulcer
Cutaneous abscess
Gas gangrene
Bite wound infection
Bones and Joints
Arthritis
Osteomyelitis
Blood
Bacteremia

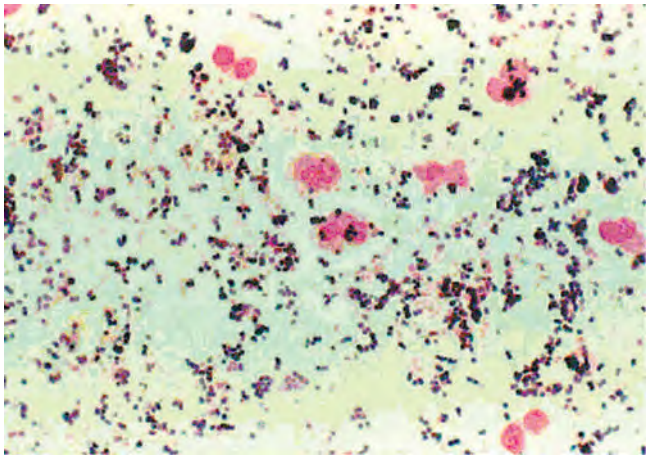

FIG. 242.1 Gram-stained specimen from a patient with meleney gangrene. This mixed infection involving *Staphylococcus aureus* and anaerobic streptococci usually occurs around surgical wounds, stomas, and cutaneous fistulas. The infection spreads slowly and often results in skin ulceration but lacks the severe systemic toxicity observed with necrotizing fasciitis. *(Courtesy Dr. Andrew Onderdonk.)*

at sites of direct bacterial contamination, although distant abscesses resulting from hematogenous spread are not uncommon with the more virulent anaerobes.

Anaerobic Infections of the Mouth, Head, and Neck

Anaerobes contribute to infections associated with periodontal disease and to disseminated infections arising from the oral cavity and spreading to adjacent structures in the head and neck[28] (see Chapter 64). The organisms isolated reflect the contiguous normal microbiota, among which *Fusobacteria, Prevotella, Porphyromonas, Bacteroides* (non-*fragilis*) and *Peptostreptococcus* spp. predominate.

Anaerobes are involved in dental infections, including pulpitis, periapical or dental abscess, and perimandibular space infection. In the gingival crevices and gums anaerobes are involved in gingivitis, periodontitis, and periodontal abscess. Formation of dental plaque, which is influenced by oral hygiene and other host factors, leads to the acquisition of pathogenic bacteria and development of these infections. Chronic periodontitis is the most common form of periodontitis. It is plaque induced and a major cause of tooth loss throughout the world. In the healthy periodontium the sparse microbiota consists mainly of gram-positive organisms, such as *Streptococcus sanguinis* and *Actinomyces* spp. In the presence of gingivitis the predominant subgingival microbiota shifts to a greater proportion of anaerobic gram-negative bacilli, with dominance of *Prevotella intermedia*. In well-established periodontitis the microbiota further increases in complexity. The predominant isolates are *Porphyromonas gingivalis, P. intermedia, Aggregatibacter actinomycetemcomitans, Treponema denticola,* and *Tannerella forsythensis.*

Infections of the periodontal area may extend into the mandible, causing osteomyelitis of the maxillary sinuses or infection of submandibular spaces.

Gingivitis may become a necrotizing ulcerative process (trench mouth, Vincent stomatitis). This disease is usually sudden in onset and is associated with tender bleeding gums, foul breath, and a bad taste. Patients may be systemically ill with fever, cervical lymphadenopathy, and leukocytosis. The infection may spread and cause destruction of bone and soft tissue or acute necrosis of the pharynx.

Noma, or cancrum oris, is a necrotizing infection of the oral mucous membranes. It is characterized by destruction of soft tissue and bone and evolves rapidly from gingival inflammation to orofacial gangrene. Noma occurs most frequently in young children ages 1 to 4 years with malnutrition or systemic disease. It occurs worldwide but is most common in sub-Saharan Africa.[29]

Perimandibular infections arise from the spread of organisms originating in the upper airways to potential spaces formed by the fascial planes of the head and neck. Two life-threatening perimandibular infections are Ludwig angina and Lemierre syndrome. Ludwig angina is a bilateral infection of the sublingual and submandibular spaces that results in marked local tissue swelling, tongue displacement, and potential airway compromise.

Lemierre syndrome,[30] usually caused by *F. necrophorum*, is an infection of the posterior compartment of the lateral pharyngeal space with secondary septic thrombophlebitis of the internal jugular vein, bacteremia, and frequent metastasis, most commonly to the lungs. Although *F. necrophorum* is the most common organism reported to cause Lemierre syndrome, other bacteria implicated include *Bacteroides*, *Eikenella*, *Streptococcus*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Proteus*, *and S. aureus.* Peritonsillar abscess is another type of deep neck space infection that is frequently caused by anaerobic bacteria, such as *F. necrophorum* and *Peptostreptococcus* spp. *F. necrophorum* has been indicated as a possible important cause of acute pharyngitis.[31]

Although anaerobic bacteria play little role in acute sinusitis, they have been implicated in chronic sinusitis in both children and adults and are found in 0% to 52% of cases, depending on the specimen collection method. These infections are usually caused by a mixture of aerobic and anaerobic bacteria. The predominant anaerobic bacteria

include *Peptostreptococcus*, *Fusobacterium*, pigmented *Prevotella* spp., and *C. acnes*.[32] Anaerobic bacteria have been isolated in a large percentage of cases of chronic suppurative otitis media in children.[33] The role of anaerobes in acute otitis media is less clear.

Central Nervous System Infections

CNS infections associated with anaerobic bacteria include brain abscess, epidural abscess, and subdural empyema. Anaerobic meningitis is rare and usually suggests a parameningeal collection or shunt infection. Anaerobic bacteria are common constituents of brain abscesses.[34,35] These organisms generally originate from the normal mouth microbiota and are most commonly associated with solitary brain abscesses originating from otorhinolaryngeal infections, such as otitis, sinusitis, or tooth infection. However, intraabdominal or pelvic infections can occasionally lead to bacteremia with an anaerobic organism that seeds the cerebral cortex. The anaerobes in such cases usually reflect the colonic or female genital tract microbiota. A single anaerobic species, or a mixture of anaerobic and aerobic bacteria, may be found in brain abscesses; prominent among the anaerobes are anaerobic or microaerophilic gram-positive cocci and *Prevotella*, *Cutibacterium*, *Fusobacterium*, *Eubacterium*, *Veillonella*, *Bacteroides* (including *B. fragilis*), and *Actinomyces*. Fig. 242.2 shows a computed tomographic scan of a left parietal brain abscess.

Pleuropulmonary Infections

Pleuropulmonary infections[36] are most commonly associated with the aspiration of frequent or large volumes of oropharyngeal material by patients with predisposing conditions, such as dysphagia due to neurologic or esophageal disorders or transiently impaired consciousness due, for example, to alcohol or drug abuse, seizures, head trauma, or cerebrovascular accident. Severe periodontal or gingival disease predisposes to anaerobic pleuropulmonary infections as well (see Chapter 64). The anaerobes most common in pleuropulmonary infections are indigenous to the oral cavity, especially the gingival crevice, and include pigmented and nonpigmented *Prevotella, Peptostreptococcus, Bacteroides* spp., and *F. nucleatum*. Many of these infections are mixed aerobic and anaerobic, and the predominant aerobes from community-acquired aspiration pneumonias are microaerophilic streptococci. A study from Japan using vigorous culture techniques in 212 patients with community-acquired lung abscess showed aerobic and microaerophilic streptococci to be the most common pathogens (60% of patients) and anaerobes to be the second most common (26%).[37] In a study on aspiration pneumonia from a long-term care facility, gram-negative bacilli were the most common isolates (49%), followed by anaerobes (16%) and *S. aureus* (12%).[38] Nosocomial aspiration pneumonia commonly involves a mixture of anaerobes and gram-negative bacilli or *S. aureus*.

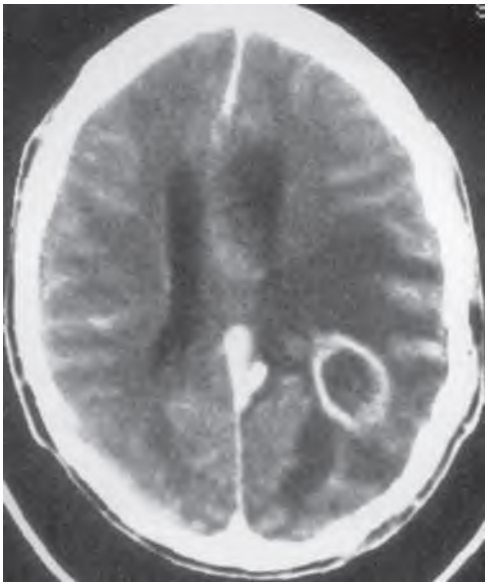

FIG. 242.2 Computed tomographic image of a left parietal brain abscess. Area outlined in *white* demarcates the walled-off abscess. *(From Mandell G, ed.* Atlas of Infectious Diseases. *Philadelphia: Churchill Livingstone; 1995.)*

Four major clinical syndromes can develop: aspiration pneumonitis, which can be complicated by necrotizing pneumonia, lung abscess, and empyema. In contrast to the abrupt course of acute pneumonias (e.g., pneumococcal pneumonia), aspiration pneumonitis has an indolent course, evolving over days or weeks instead of hours. Patients usually present with subacute and chronic pulmonary symptoms and manifestations of chronic disease, including weight loss and anemia. Chills are uncommon. The lobes of the lung that are affected are those that were dependent, according to the position of the patient when the aspiration occurred. The sputum is not foul smelling initially but can become malodorous with prolonged infection, and Gram stain reveals a mixed flora. Sputum samples are not reliable for culture because they contain the normal oral flora, but cultures of samples obtained by transtracheal or transthoracic aspiration, which currently are rarely used, may be of value. Protected brush or bronchoalveolar lavage samples obtained by bronchoscopy are controversial because of possible contamination and difficulty associating specific microbes with disease etiology.

Necrotizing pneumonia is characterized by the development of many small abscesses within the pulmonary parenchyma. Lung abscesses most often arise secondary to the development of periodontal disease, and, as would be expected, oral anaerobes predominate. Empyemas are a result of long-term anaerobic pulmonary infection complicated by bronchopleural fistula, foul-smelling sputum, and pleuritic chest pain.

Intraabdominal Infections

Intraabdominal infections, mainly peritonitis (generalized or localized) and abscesses, are usually polymicrobial and result from a breach in the continuity of the mucosal surface and spillage of the normal microbiota into the sterile peritoneal cavity. The cause of the breach can be appendicitis, diverticulitis, neoplasm, inflammatory bowel disease, surgery, or trauma. In infections originating from colonic sites, specimens yield, on average, four to six species, with a predominance of coliforms, anaerobes, and streptococci/enterococci. The most common isolates are *Escherichia coli* and *Bacteroides* spp., among which *B. fragilis* is predominant (see Chapter 74). Other anaerobes commonly isolated from this type of infection include *Peptostreptococcus, Prevotella,* and *Fusobacterium* spp. The involvement of clostridia can lead to severe infections. The dominance of four to six bacterial species out of more than 500 different colonic mucosal species is related to both virulence factors of these species and the inability of clinical laboratories to grow in culture many other species residing in the colonic mucosa.

Disease originating from proximal bowel perforation reflects the microbiota of that site, with a predominance of aerobic and anaerobic gram-positive bacteria and *Candida*. Anaerobic bacteria have been implicated in enterocolitis (typhlitis), an infection of the cecum or the entire bowel in the setting of neutropenia. *Clostridium septicum,* other clostridia, and mixed anaerobes have also been implicated.

B. fragilis has been associated with watery diarrhea in case-control studies of children with undiagnosed diarrheal disease. Enterotoxin-producing strains are more prevalent in patients with diarrhea than in control groups. An etiologic relationship between enterotoxin-producing *B. fragilis* strains and diarrhea has been suggested. [39]

Female Genital Tract Infections

The female genital tract is a major reservoir for anaerobes, which outnumber aerobes at this site by 10:1. Anaerobes are encountered in nearly all infections that are not caused by sexually transmitted agents, including vulvar and Bartholin gland abscesses, pelvic inflammatory disease and adnexal abscesses, septic pelvic thrombophlebitis, intrauterine contraceptive device–associated infection, endometritis, amnionitis, septic abortion, and postoperative or postpartum infections (see Chapter 109). The major isolates from these infections are *P. bivia*, *P. disiens,* and *Peptostreptococcus*, *Porphyromonas*, and *Clostridium* spp. *Actinomyces* and *Eubacterium* spp. are isolated from infections associated with intrauterine devices. Like intraabdominal infections, most infections of the female genital tract are of mixed etiology, involving both anaerobes and aerobes.

Bacterial vaginosis, a disease process in which anaerobes predominate, is characterized by malodorous discharge and inflammation. Although the etiology is not clear, a change in bacterial ecology, with consequent overgrowth of certain bacterial species that replace the *Lactobacillus*-dominated normal microbiota, has been suggested. The anaerobic bacteria involved include *Gardnerella vaginalis, Prevotella*, *Mobiluncus*, and *Peptostreptococcus* spp. A study based on 16S rRNA identification found other anaerobes that were more common in cases than in controls: *Atopobium, Leptotrichia, Megasphaera,* and *Eggerthella*.[40]

Skin and Soft Tissue Infections

Anaerobic infections in the skin and soft tissue can be caused by the cutaneous anaerobic microbiota, mainly *Peptostreptococcus,* but are most often caused by contamination with the flora from adjacent mucosal surfaces.[41] Examples include infected sebaceous and pilonidal cysts, paronychia, breast abscesses, surgical wound infections, human or animal bites, diabetic foot ulcers, and decubitus ulcers. Aspirates from wounds and subcutaneous tissue infections and abscesses below the waist (e.g., decubitus ulcer and diabetic foot infection) yield colonic mixed microbiota, including aerobes and anaerobes, mainly the *B. fragilis* group and *Clostridium* spp., together with Enterobacteriaceae and *Enterococcus* spp. Infections around the oropharynx or infections that originate from that site (paronychia, bites) harbor oral microbiota, such as *Prevotella, Porphyromonas, Fusobacterium,* and *Peptostreptococcus* spp. Anaerobes can also be found in deep soft tissue infections, such as necrotizing fasciitis, synergistic cellulitis, crepitant cellulitis, and gas gangrene, usually as part of a mixed anaerobic/aerobic etiology. This type of infection usually occurs at sites that can be contaminated from oral secretions or feces; the disease can spread rapidly and can be destructive. Gas may be found in the infected tissues. The major pathogens in these deep infections include a combination of aerobes and anaerobes, mainly group A β-hemolytic streptococci, clostridia, peptostreptococci, and *Bacteroides* spp. Fournier gangrene, a form of cellulitis that involves the scrotum, perineum, or anterior abdominal wall and results in extensive loss of skin, may require aggressive surgical intervention.

Bone and Joint Infections

Many patients with osteomyelitis due to anaerobic bacteria have evidence of an anaerobic infection elsewhere in the body. Infected adjacent soft tissue sites are the source of the organisms involved in the osteomyelitis. Examples are diabetic foot ulcers and decubitus ulcers that may be complicated by mixed aerobic/anaerobic osteomyelitis. Cranial and facial bone osteomyelitis is usually caused by oral microbiota that spread from a contiguous soft tissue source or from sinus, ear, or dental infection. Hematogenous seeding of bones with anaerobic bacteria is uncommon. The predominant anaerobes in osteomyelitis of all sites are *Bacteroides* and *Peptostreptococcus* spp. *Prevotella*, *Fusobacterium*, and *Porphyromonas* spp. are prevalent in skull and bite infections. *Clostridium* spp. are found in osteomyelitis associated with wound contamination after trauma or exposure to gut microbiota.

Most cases of anaerobic arthritis, in contrast to anaerobic osteomyelitis, involve a single isolate, and most cases are secondary to hematogenous spread, trauma, or a prosthetic joint. The predominant anaerobes in arthritis are the *B. fragilis* group, *Fusobacterium* spp., and *Peptostreptococcus* spp.[42]

Cutibacterium is occasionally identified in infections involving prosthetic devices, especially shoulder devices.[43]

Anaerobic Bacteremia

Anaerobes are an important cause of bloodstream infections and have accounted for approximately 5% (range, 0.5%–12%) of cases of clinically significant bacteremia, but the rate of anaerobic bacteremia (AB) decreased from the 1970s through the early 1990s. Recent reports present conflicting data regarding rates of AB. A study from the Mayo Clinic compared three periods (1993–96, 1997–2000, 2001–04) and found a 74% increase in the mean incidence of AB.[44] In contrast, a report from Switzerland compared two periods (1997–2001 and 2002–06) and found decreases in both the number of anaerobe-positive blood cultures and the proportion of all blood culture isolates that were anaerobes.[45]

The majority of cases of AB are due to gram-negative bacilli, mainly from the *B. fragilis* group; *B. fragilis* is the anaerobe most commonly isolated from these infections (60%–80% of cases).[46] Reported fatality rates are high, ranging from 25% to 44%, and *B. fragilis* group bacteremia contributes to morbidity and mortality.[47] Other species causing AB include *Clostridium* spp. (10%), *Peptostreptococcus* spp. (10%), and *Fusobacterium* spp. (5%). AB is usually secondary to an infectious process that has emanated from an intraabdominal source, the female genital tract, the respiratory tract, or soft tissue. Debilitating diseases such as malignancies, diabetes, organ transplantation, and abdominal and pelvic surgeries are among the predisposing factors for AB.[48]

In a retrospective nested case-control study, diabetes was found as a risk factor for AB when the source of bacteremia was unknown.[49]

PATHOGENESIS OF ANAEROBIC INFECTIONS

Infections caused by anaerobes are generally a result of the breakdown of a mucosal barrier and the subsequent leakage of the indigenous polymicrobial flora into previously sterile closed spaces or tissue. The introduction of many species of bacteria into otherwise sterile sites leads to a polymicrobial infection in which certain organisms predominate. Tissue ischemia, trauma, surgery, perforated viscus, shock, and aspiration provide anaerobes with the opportunity to penetrate mucosal barriers and enter tissue with a lowered oxidation-reduction potential conducive to proliferation. Three major factors are involved in the pathogenesis of anaerobic infections: virulence factors of the organisms, bacterial synergy, and mechanisms of abscess formation. The predominant gram-negative anaerobes in these infections include *B. fragilis*, *Prevotella*, *Fusobacterium*, and *Porphyromonas* spp. Although some of these organisms are numerically dominant in the normal microbiota, others make up a much smaller proportion (e.g., *B. fragilis*, 0.5%); thus their predominance among clinical isolates indicates that they possess one or more virulence factors that enhance their ability to cause disease. Typically, virulence factors associated with anaerobes confer the ability to evade host defenses, adhere to cell surfaces, produce toxins and enzymes, or display surface structures, such as capsular polysaccharides, that contribute to pathogenic potential. Table 242.4 lists some of the virulence factors associated with anaerobic organisms commonly isolated from clinical infections.

TABLE 242.4 Colonization Factors Associated With Pathogenic Anaerobes That Contribute to Virulence of Medically Important Anaerobes

Bacteroides Fragilis
Capsular polysaccharides
Neuraminidase
Proteases
Enterotoxin
Hemagglutinin
Porphyromonas Gingivalis
Proteases (gingipains)
Lipopolysaccharides
Capsule
Hemolysin
Fusobacterium Necrophorum
Leukotoxin
Hemolysin
Lipopolysaccharides
Phospholipase
Proteases
Fusobacterium Nucleatum
Lipopolysaccharides
Adhesins
Proteases
Leukotoxin
***Prevotella* Spp.**
Lipopolysaccharides
Proteases

The ability of anaerobic bacteria to act synergistically during polymicrobial infection contributes to the pathogenesis of anaerobic infections. The phenomenon of microbial synergy in these infections remains poorly characterized. It has been postulated that facultative organisms function in part to lower the oxidation-reduction potential in the microenvironment and that this change allows the propagation of obligate anaerobes. Additional studies indicate that anaerobes can produce compounds such as succinic acid and short-chain fatty acids that inhibit the ability of phagocytes to clear facultative organisms. Studies in experimental models demonstrate that facultative and obligate anaerobes synergistically potentiate abscess formation.

Although it constitutes only 0.5% to 1% of the normal colonic microbiota, *B. fragilis* is the anaerobe most commonly isolated from intraabdominal infections and bacteremia. The high frequency of abscess formation associated with *B. fragilis* led to studies of this organism's pathogenic potential in relevant animal models of disease. In an animal model of intraabdominal sepsis the capsular polysaccharide was identified as the major virulence factor of *B. fragilis;* this polymer plays a specific, central role in the induction of abscesses.[50] A series of detailed biologic and molecular studies of this virulence factor showed that *B. fragilis* produces at least eight distinct capsular polysaccharides,[51] far more than the number reported for any other encapsulated bacterium. *B. fragilis* can exhibit a wide array of distinct surface polysaccharide combinations by regulating the expression of these different capsules in an on-off manner through the reversible inversion of DNA segments containing the promoters for their expression. Structural analysis of two of these polysaccharides, PSA and PSB, revealed that each polymer consists of repeating units with positively charged free amino groups and negatively charged groups. This structural feature is rare among bacterial polysaccharides, and the ability of PSA and, to a lesser extent, PSB to induce abscesses in animals depends on this zwitterionic charge motif.[52]

Mechanistic studies of the pathogenesis of intraabdominal abscess formation by *B. fragilis* revealed a multifunctional role for its capsular polysaccharides in this process. PSA was found to activate host $CD4^+$ T cells and promote the release of interleukin-17 (IL-17), interferon-γ, and chemokines[53] through a Toll-like receptor 2–dependent mechanism.[54] In addition, the capsule induces the release of the proinflammatory cytokines tumor necrosis factor-α and IL-1β from peritoneal macrophages. These cytokines potentiate the increase of cell adhesion molecules, such as intracellular adhesion molecule-1 on mesothelial cell surfaces, which in turn leads to an increase in the binding of neutrophils to these cells and initiates abscess formation.[55] The capsules of *B. fragilis* also facilitate binding of the organism to mesothelial cells lining the surface of the peritoneal cavity.

B. fragilis produces other virulence factors that allow it to predominate in disease. Although the lipopolysaccharide (LPS) of *B. fragilis* possesses little biologic activity, this organism synthesizes pili, fimbriae, and hemagglutinins that aid in attachment to host cell surfaces. In addition, *Bacteroides* spp. produce many enzymes and toxins that contribute to pathogenicity. Enzymes such as neuraminidase, protease, glycoside hydrolases, and superoxide dismutases are all produced by *B. fragilis*. An enterotoxin termed *B. fragilis* toxin (BFT) has also been identified in *B. fragilis*. This toxin is a metalloprotease that is cytopathic for intestinal epithelial cells and induces fluid secretion and tissue damage in ligated intestinal loops of experimental animals. Strains producing the toxin have been recovered from patients with diarrhea and from healthy controls. An association of BFT-positive *B. fragilis* with clinical episodes of diarrhea in children and adults has been suggested. There is evidence from mouse models that enterotoxin-producing strains of *B. fragilis* may play a role in colon carcinoma.

A prominent etiologic agent in adult periodontitis, *P. gingivalis,* relies on a broad range of virulence factors to cause disease.[56] These include extracellular proteases (cysteine proteinases) that can cause attachment, degradation, or cleavage of host cell proteins and surface receptors and can modulate the host immune response; adhesins such as fimbriae and hemagglutinins; and a putative invasin (halo acid dehalogenase family phosphoserine phosphatase). The capsular polysaccharide of *P. gingivalis* is a potent virulence factor that facilitates the spread of infection in mice to an extent greater than that seen with unencapsulated strains. The LPS of *P. gingivalis* has strong proinflammatory activity and has been implicated in the initiation and development of periodontal disease. *P. gingivalis* has been shown to invade and replicate within host cells, a mechanism that may facilitate its spread. It also evades the host immune response through modulation of innate immune function.

F. necrophorum causes numerous necrotic conditions and human oral infections. Toxins such as leukotoxin, endotoxin, and hemolysin have all been implicated as virulence factors, with leukotoxin and endotoxin playing an important role in the pathogenesis of disease. *F. nucleatum* has been isolated frequently from cases of periodontitis and contributes significantly to gingival inflammation. This organism coaggregates with other oral bacteria to promote attachment to plaque; in addition, it produces several adhesins that facilitate attachment. Both *F. nucleatum* and *F. necrophorum* produce a potent LPS that is responsible for the release of numerous proinflammatory cytokines and other inflammatory mediators that may play a pathogenic role in periodontal disease; in addition, this LPS presumably accounts for the severity of illness in Lemierre syndrome.

Virulence factors associated with *Prevotella* spp. are poorly defined. The organisms' ability to interact with other anaerobes has been reported. Among their prominent virulence traits is the production of proteases and metabolic products such as volatile fatty acids and amines. This group of organisms is particularly noted for secretion of immunoglobulin A (IgA) proteases. The degradation of IgA produced by mucosal surfaces allows *Prevotella* to evade this first line of host defense. *P. intermedia* can invade oral epithelial cells, and antibody specific for fimbriae from this organism inhibits invasion.

Among the most clearly identified virulence factors for anaerobic bacteria are the exotoxins produced by clostridial species, including botulinum toxins, tetanus toxin, *C. difficile* toxins, and five toxins produced by *C. perfringens* (as well as many other clostridial species). These are among the most virulent bacterial toxins known, as demonstrated in mouse lethality assays.

DIAGNOSIS OF ANAEROBIC INFECTIONS

Anaerobic organisms can be difficult to culture, and their identification can be expensive, time consuming, or even misleading because of confusion with normal microbiota in some cases. Therefore many anaerobic infections are diagnosed because the presence of anaerobes is suspected. There are few clinical clues to the probable presence of anaerobic bacteria at infected sites. Infections at various sites, especially those proximal to mucosal surfaces with indigenous anaerobic microbiota, are indicative of the presence of anaerobes, particularly in the GI tract, female genital tract, or oral cavity. Anaerobes are often associated with tissue necrosis and abscess formation at all anatomic locations, including cerebral, dental, peritonsillar, lung, intraabdominal, tubo-ovarian, and cutaneous sites.

A foul odor or gas is highly suggestive of anaerobes as well, although the absence of these factors does not rule out anaerobic infection. In fact, a putrid odor may be a relatively late feature and develops in only approximately one-third to one-half of patients. Furthermore, gas in tissue can sometimes be produced by aerobic bacteria such as *E. coli*. A patient's failure to respond to antibiotics that are not active against anaerobes suggests anaerobic infection. Because anaerobic infections are often polymicrobial, Gram stain of exudates showing a polymicrobial flora and organisms with morphologic features of anaerobes is indicative of anaerobic infection. For example, *Bacteroides* spp. are small, delicate gram-negative bacilli; *F. nucleatum* is a fusiform bacteria with pointed ends; *F. necrophorum* is a long, "ropy" gram-negative bacilli, and *Clostridium* spp. are large "boxcar"-like gram-positive bacilli. By contrast, *Peptostreptococcus* cannot be distinguished from aerobic gram-positive cocci on the basis of Gram stain appearance.

When samples from suspected anaerobic infections are cultured, it is imperative that they be properly collected and transported. Samples should be collected so as to avoid contamination by indigenous flora of mucosal surfaces. The optimal specimens are normally sterile fluids (e.g., blood, pleural, and peritoneal fluids, and aspirates) or biopsy samples from normally sterile sites. In general, liquids or tissues are preferable to swab specimens. The optimal way to transport specimens is by immediate delivery to permit prompt microbiologic processing

or with anaerobic transport tubes. Suitable commercially available anaerobic-transport media should be used. Although many anaerobes are aerotolerant, exposure to oxygen, even for the briefest period, can interfere with culture of some organisms. Selective and nonselective media should be used for culture to identify the clinically relevant anaerobes. It is also important to remember that prior antibiotic therapy reduces cultivability of these bacteria. Specimens should be processed as quickly as possible and handled appropriately in the clinical microbiology laboratory; they should be subjected to Gram staining, and the results should be compared with those of culture. It is not uncommon for specimens to yield no growth on culture but for numerous organisms (both gram-positive and gram-negative) to be evident on Gram staining, a result suggesting that anaerobic organisms are present. Sterile pus may indicate anaerobic infection with failed collection or identification methods. Recent advances in direct detection of anaerobes from clinical samples include 16S rRNA gene–based methods, DNA hybridization, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Biotyper, multiplex polymerase chain reaction, and oligonucleotide array technologies.[57]

TREATMENT OF ANAEROBIC INFECTIONS AND ANTIBIOTIC RESISTANCE

Successful therapy for anaerobic infections generally involves the administration of appropriate antimicrobial agents combined with surgical management or percutaneous drainage. Because anaerobic infections can cause severe tissue damage or result in abscess formation, débridement of necrotic tissue, drainage of abscesses, restoration of airspaces, resection, maintenance of blood supply, or a combination of these interventions is necessary.

The antibiotics used to treat anaerobic infections should be active against both aerobic and anaerobic organisms because many of these infections are of mixed etiology. Antibiotic regimens are usually selected empirically on the basis of the type of infection, the species of organisms usually present in such cases, Gram stain results, and knowledge of antimicrobial resistance patterns. Other factors influencing the selection of antibiotics include the need for bactericidal activity and for penetration into certain organs (such as the brain), the risk of toxicity, and consideration of the impact on the normal microbiota. Antibiotic susceptibility testing of anaerobic bacteria is rarely performed in clinical laboratories because of inadequate anaerobic culture techniques, difficulty in obtaining results within a useful time frame, and poor quality control of in vitro susceptibility results. It is accepted that testing is important for patients with serious or prolonged infections or in cases in which antibiotics have not had an impact. Testing is also helpful in monitoring the activity of new drugs and recording current resistance patterns among anaerobic pathogens. A correlation between the antimicrobial resistance of an anaerobic pathogen and a poor clinical outcome has been reported in several retrospective trials. A study of antibiotic-treated patients with *Bacteroides* isolates from blood found mortality rates of 45% among those whose isolates were deemed resistant to the agent used and 16% among those whose isolates were deemed sensitive.[58] Notwithstanding this report, antibiotic regimens are still often selected empirically with good results.

The antibiotics with the greatest activity against nearly all anaerobic bacteria include carbapenems, β-lactam/β-lactamase inhibitor combinations, metronidazole, and chloramphenicol (Table 242.5). Antibiotic resistance is increasingly reported among anaerobic bacteria.[59] Resistance rates vary widely among different geographic regions and institutions. The major changes have involved the activity of clindamycin, cephamycins, and moxifloxacin against *B. fragilis* and related strains (*P. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*). Of interest are the higher resistance rates found among the "other *Bacteroides*" (non–*B. fragilis*) than in *B. fragilis,* even though the "other *Bacteroides*" are less frequently isolated from clinical cases.

Penicillin G is the drug of choice when the infecting strains are susceptible. Most *Clostridium* strains and *Peptostreptococcus* spp. remain susceptible to penicillin. However, the medically important *Bacteroides* spp. are typically resistant to penicillin G. Other strains that may exhibit resistance to penicillin include pigmented and nonpigmented *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., and microaerophilic streptococci. Anaerobes manifest three major mechanisms of resistance to β-lactam antibiotics: inactivating enzyme production (mainly β-lactamases such as penicillinase and cephalosporinase), low-affinity penicillin-binding protein expression, and decreased permeability through porin channel alteration. The production of β-lactamases is the most common mechanism among anaerobes, especially among the *B. fragilis* group; some other *Bacteroides* spp.; and *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp.

Penicillin G, ampicillin, and amoxicillin are equally active against anaerobic strains that do not produce β-lactamase, but the semisynthetic penicillins (methicillin, nafcillin, and oxacillin or dicloxacillin) have unpredictable activity and frequently are inferior to penicillin G. The semisynthetic penicillins—carboxypenicillins (carbenicillin, ticarcillin) and ureidopenicillins (piperacillin, azlocillin, mezlocillin)—generally are administered in high doses and display good activity against most anaerobes in these concentrations; however, up to 30% of *B. fragilis* strains are resistant to these agents.

Cephalosporins have limited use because many anaerobes, including the *B. fragilis* group and many *Prevotella*, *Porphyromonas*, *and Fusobacterium* spp., produce cephalosporinases. The cephalosporinases has little or no activity against the cephamycins cefoxitin and cefotetan, which display greater activity against anaerobic organisms, Approximately 85% of *B. fragilis* isolates are susceptible to cefoxitin, but the other *B. fragilis* group species are more resistant.[59] Cefotetan is less effective than cefoxitin against *B. fragilis* and other members of the *B. fragilis* group.

β-Lactam/β-lactamase inhibitor antibiotic combinations, such as piperacillin-tazobactam and ampicillin-sulbactam, are usually a good option against β-lactamase–producing anaerobes, including the *B. fragilis* group. Resistance rates reported from most countries are still low: 0.5% to 3% in the United States, 3% to 10% in Germany, and 1% to 8% in Argentina. However, up to 48% of *B. fragilis* isolates in Taiwan were found to be nonsusceptible to ampicillin-sulbactam, and a significant increase in resistance to ampicillin-sulbactam among other *Bacteroides* spp., *Prevotella* spp., and *Fusobacterium* spp. was found as well.[60]

Ceftazidime-avibactam, one of the newer β-lactam/β-lactamase inhibitor antibiotic combinations, has limited action against anaerobic bacteria. A study of 396 samples demonstrated the inefficiency of this combination against anaerobes, such as *B. fragilis, Fusobacterium* spp., *Prevotella* spp., and *Porphyromonas* spp.[61] For treatment of intraabdominal infections, ceftazidime-avibactam should be combined with metronidazole.

Carbapenems (ertapenem, doripenem, meropenem, and imipenem) are equally active against anaerobes, with <1% of *B. fragilis* strains showing resistance in the United States and Europe. Higher rates of nonsusceptibility are being reported from some countries (5% in Germany, 8% [to doripenem] in Canada, and 7%–12% in Taiwan).

Metronidazole is active against gram-negative anaerobes, including the *B. fragilis* group; resistance is rare (<1%) in both Europe and the United States but has been reported. Resistance to metronidazole is more prevalent among gram-positive anaerobes, including *C. acnes, Actinomyces* spp., lactobacilli, and anaerobic streptococci. This antibiotic is well tolerated, reaches significant levels in serum, and penetrates abscesses well.

Clindamycin is active against many anaerobes. Rates of resistance to clindamycin among the *B. fragilis* group have increased in the United States from 3% in 1982 to 16% in 1996 and 26% in 2000, with rates as high as 40% to 50% in some series. Resistance has also increased in many non-*Bacteroides* anaerobes. Up to 10% resistance was found in *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus spp*.

C. acnes isolates have also become more resistant to clindamycin, especially in association with previous therapy for acne.

Fluoroquinolones such as moxifloxacin have the potential to treat mixed aerobic/anaerobic infections. However, a survey in the United States found 38% resistance to moxifloxacin among the *B. fragilis* group; in Europe 14% of isolates were resistant, and in Taiwan 7% to 25% of anaerobes isolated from blood cultures were resistant to moxifloxacin.

The macrolides erythromycin, azithromycin, and clarithromycin have moderate-to-good in vitro activity against anaerobic bacteria other than the *B. fragilis* group and other gram-negative anaerobes, but the published clinical experience is limited.

TABLE 242.5 Antimicrobial Agents Effective Against Medically Important Anaerobes

ANTIMICROBIAL AGENT	COMMENT
Nearly Always Active	
Carbapenems (imipenem, meropenem, doripenem)	Resistant to most *Bacteroides* β-lactamases
Metronidazole	Bactericidal against most gram-negative anaerobic strains; inactive against *Cutibacterium* spp., *Actinomyces* spp., peptostreptococci, and microaerophilic streptococci such as *Streptococcus anginosus*
β-Lactam/β-lactamase inhibitor combination (ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid)	Good option against β-lactamase–producing anaerobes, including the *Bacteroides fragilis* group; low resistance rates
Chloramphenicol	Despite excellent in vitro activity against all clinically important anaerobes, this drug is less desirable than other active drugs because of documented clinical failures and bone marrow toxicity
Variably Active	
Clindamycin	Resistance among the *B. fragilis* group has increased in recent years, up to 60% of strains in some reports. Clindamycin no longer recommended for complicated intraabdominal infections
Cephamycins (cefoxitin, cefotetan)	Variable and increasing resistance. Cefotetan no longer recommended for complicated intraabdominal infections
High-dose antipseudomonal penicillins (carbenicillin, ticarcillin, piperacillin, azlocillin, mezlocillin)	Good activity against most anaerobes; however, up to 30% of *B. fragilis* strains are resistant to these agents
Tigecycline	Active against nearly all anaerobes, including *Bacteroides* spp. Resistance has been reported
Variably Resistant	
Penicillin	Inactive versus some or most penicillinase-producing anaerobes, including most of *B. fragilis* group and many strains of *Prevotella*. Most *Clostridium* strains and *Peptostreptococcus* spp. are susceptible
Cephalosporins (excluding cephamycins)	Limited utility because many anaerobes, including the *B. fragilis* group and many *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp., produce cephalosporinases
Tetracyclines (doxycycline and minocycline)	Limited use because of significant resistance
Vancomycin	Active against gram-positive anaerobes; inactive against gram-negative anaerobes
Macrolides	Moderate-to-good in vitro activity against anaerobic bacteria other than the *B. fragilis* group and other gram-negative anaerobes; however, published clinical experience limited
Fluoroquinolones (moxifloxacin)	*B. fragilis* group shows increasing resistance, and moxifloxacin no longer recommended in the IDSA guidelines for complicated intraabdominal infections
Resistant	
Aminoglycosides	—
Trimethoprim-sulfamethoxazole	—
Monobactams (aztreonam)	—

IDSA, Infectious Diseases Society of America.

Among the tetracyclines, doxycycline and minocycline are of limited use because of significant resistance. Tigecycline, a glycylcycline, is active against anaerobic bacteria, including most *Bacteroides* spp., peptostreptococci, and *Cutibacterium, Prevotella*, and *Fusobacterium* spp. Tigecycline has been used as a single-agent treatment for complicated intraabdominal infections; however, resistance (≈6%) among *Bacteroides* and non-*Bacteroides* spp. has been reported.

Despite excellent in vitro activity against all clinically important anaerobes, chloramphenicol is less desirable than other active drugs for the treatment of anaerobic infection because of documented clinical failures and bone marrow toxicity.

Anaerobic bacteria are intrinsically resistant to aminoglycosides and trimethoprim-sulfamethoxazole.

As mentioned previously, resistance among some anaerobes has increased significantly over the past 3 decades. Recently, cases of multidrug-resistant *B. fragilis* have been reported; isolates were resistant to metronidazole, carbapenems, piperacillin-tazobactam, and clindamycin, among other agents.

In clinical situations specific regimens must be tailored to the initial site of infection. Antibiotic treatment for intraabdominal infections needs to be directed against *Bacteroides* spp. and the gram-negative aerobic flora of the bowel. Single agents suitable for patients with mild-to-moderate community-acquired intraabdominal infections include the carbapenems and β-lactam/β-lactamase inhibitor combinations, such as piperacillin-tazobactam or ticarcillin-clavulanate. The Surgical Infection Society/Infectious Diseases Society of America 2010 guidelines also list cefoxitin, moxifloxacin, and tigecycline as options, but these agents should usually be avoided because of substantial rates of in vitro resistance to cefoxitin and fluoroquinolones among *Bacteroides* spp. and coliforms and concern about greater mortality associated with tigecycline than with other antibiotics for various infections, including intraabdominal infections. Moxifloxacin may still be cautiously used for intraabdominal infections, provided that the patient has mild-to-moderate disease and has not been exposed to fluoroquinolones recently.

A two-drug regimen is an alternative, with one drug active against coliforms and the other against anaerobes (e.g., a third-generation cephalosporin or a quinolone with metronidazole). In addition, if the clinician suspects that gram-positive facultative organisms, such as enterococci, are involved, therapeutic regimens should include ampicillin or vancomycin. Although clindamycin and cefotetan were previously considered acceptable options for intraabdominal infections involving anaerobes, these drugs are no longer recommended because of escalating rates of resistance in the *B. fragilis* group. (Ampicillin-sulbactam is not recommended because of high rates of resistance among community-acquired *E. coli*, not because of resistance in anaerobic bacteria).[62]

Mixed aerobic/anaerobic infections of oral origin must include drugs active against both the gram-positive aerobic microbiota and the anaerobic flora of the mouth, which usually does not include the *B. fragilis* group. β-Lactam drugs (penicillins and cephalosporins) alone are not suitable because of β-lactamase production by anaerobic strains

that are usually isolated from infections originating above the diaphragm (*Fusobacterium, Prevotella,* and *Peptostreptococcus*). Suitable regimens for these infections include clindamycin, β-lactam/β-lactamase inhibitor combinations, or penicillin together with metronidazole.

The failure of antibiotic therapy against an anaerobic infection should prompt consideration of surgical drainage or débridement of the infected site. In addition, the possibility of coinfection with one or more drug-resistant aerobic organisms should be considered. In these situations isolation of the organisms should be attempted to determine antibiotic susceptibility.

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243 *Clostridioides difficile* (Formerly *Clostridium difficile*) Infection

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SHORT VIEW SUMMARY

Definition
- *Clostridioides difficile* infection (CDI) is an acute diarrheal illness due to colitis that is most often preceded by antimicrobial use and is caused by toxins of an anaerobic spore-forming bacterium, *Clostridioides difficile* (formerly *Clostridium difficile*).

Epidemiology
- *C. difficile* is found in water, soil, meats, and vegetables and is particularly common in health care environments, where the spores are difficult to eradicate. Patients are exposed to and ingest *C. difficile* spores in the health care setting from contact with the environment or health care workers who do not practice good hand hygiene.
- If the patient has taken antibiotics recently, the normal microbiota is likely to be disrupted, and in this disrupted microbiota the ingested spores, which are resistant to stomach acid, can germinate.

Pathogenesis
- Vegetative cells proliferate in the colon, producing two major toxins: A (primarily an enterotoxin) and B (primarily a cytotoxin). The two toxins produce a marked neutrophilic inflammatory response in the colon, resulting in diarrhea, erosion of the mucosa, and formation of pseudomembranes composed of necrotic cells and proteinaceous material that extends over the mucosal surface.
- Recurrence of diarrhea following treatment occurs in 25% of patients and requires re-treatment.

Microbiology
- *C. difficile* are obligate gram-positive anaerobic bacteria that can survive in environments under aerobic conditions by forming spores.
- The majority of strains produce two toxins, A and B, thought to be responsible for many of the disease characteristics. A third toxin, binary toxin, is produced by recent epidemic strains that have caused outbreaks with increased severity and mortality.
- Some strains of *C. difficile* lack the genes for toxin production and are nontoxigenic, and are not thought to cause CDI.

Diagnosis
- Diagnosis of CDI is based on the presence of clinical symptoms (usually defined as >3 watery, loose, or unformed stools within a period of 24 hours or less) coupled with a diagnostic test (usually of a stool specimen) that detects the presence of either the *C. difficile* organism or its toxin genes, or detection of *C. difficile* toxin using an enzyme immunoassay or cell cytotoxin assay.

Therapy
- The first steps in effective treatment are to stop the offending antibiotic and provide fluid and electrolyte support, if needed.
- Specific treatment consists of vancomycin or fidaxomicin orally for mild-to-moderate disease (metronidazole may also be used but is less effective), vancomycin or fidaxomicin orally for severe disease (white blood cell count >15,000/mL, or creatinine >1.5 × baseline), and vancomycin orally or by nasogastric tube plus intravenous metronidazole for severe complicated (also called fulminant) CDI that presents with hypotension, shock, ileus, or megacolon.
- Patients with recurrent CDI may benefit from oral vancomycin in a taper and pulse dose, fidaxomicin, or the monoclonal antibody bezlotoxumab. Multiple CDI recurrences are effectively treated by fecal microbiota transplant (FMT).

Prevention
- Prevention in the health care environment is focused on preventing patient exposure to spores of *C. difficile* by utilizing isolation, cohorting, gloves and gowns, and hand washing (alcohol rubs are ineffective against spores). Bleach is used in the environment to eradicate spores.
- It is exceedingly difficult to prevent spore exposure, so antimicrobial stewardship programs to reduce unnecessary antimicrobial use are extremely effective in limiting the number of susceptible patients.
- A number of new preventive approaches are undergoing clinical investigation, including vaccines to induce antibodies to toxins A and B, and biotherapeutics that are live bacterial organisms such as FMT or spores of nontoxigenic *C. difficile*.

The administration of antibiotics can be complicated by a number of unintended consequences, among which gastrointestinal side effects are quite common. Gastrointestinal symptoms occur in up to 25% to 50% of patients depending on the specific antimicrobial agent, patient population, and epidemiology.[1] While the majority of these side effects are mild, consisting of minor antibiotic-associated diarrhea (AAD) without systemic signs of illness, some patients can develop frank colitis and severe clinical manifestations, including toxic megacolon, intestinal perforation, sepsis, and death. Infection with *Clostridioides difficile* (formerly *Clostridium difficile*) is thought to be responsible for about 25% of all cases of AAD and is the underlying etiology in nearly all cases of severe disease and pseudomembranous colitis (PMC).

While Koch's postulates were fulfilled for *C. difficile* 40 years ago,[2] we still have an incomplete understanding of how disruption of the normal indigenous gut microbiota leads to *C. difficile* colonization and frank disease. Additionally, while adequate antimicrobial therapy for *C. difficile* infection was described shortly after the etiologic role for the pathogen was established,[3] a recent increase in the apparent severity, prevalence, and recurrence of *C. difficile* infection (CDI)[4,5] has prompted additional interest in understanding the pathogenesis of this infection.

HISTORICAL OVERVIEW

Prior to 1978 the disease pseudomembranous colitis and the organism *Clostridioides difficile* had never been linked. PMC was described in humans by Finney in 1893,[6] and in animals in 1943 by Hambre and colleagues, following administration of penicillin that resulted in rapid animal death.[7] PMC occurred in patients prior to the antibiotic era, perhaps consistent with the lack of prior antibiotic use in a small subset of current CDI patients.[8] However, it was the antibiotic era that was accompanied by an increase in PMC, particularly in association with use of lincomycins.[9,10] It was the publication by Tedesco and coworkers[10] in 1974 that started the search for a cause for PMC linked to clindamycin use, at the time called *clindamycin-associated colitis*. Of

200 clindamycin-treated patients, 21% were found to have diarrhea and nearly half of those (10%) were found to have PMC, including 38% of patients who inexplicably had onset of diarrhea after clindamycin had been stopped.

After 4 years of intensive research, in 1977 and 1978 a succession of papers leading to the identification of *C. difficile* and its toxins as the cause of clindamycin-associated colitis or PMC and of vancomycin as an effective treatment were published. The laboratories of Larson in the United Kingdom, and Bartlett, Fekety, and Finegold in the United States were largely responsible for these discoveries, using the hamster as an essential animal model for CDI.[11–17]

In retrospect, the rapidity and thoroughness of the CDI research discoveries of the 1970s may have engendered an early perception that there was little else to learn. This may have led to a clinical perception of CDI as a nuisance disease that was not even monitored as a health care–associated infection in many hospitals. This perception of CDI changed markedly in the early 21st century when CDI epidemics in North America and Europe with high mortality occurred that were caused by a previously little-known *C. difficile* strain that has been variously described as North American pulsed-field gel electrophoresis type 1 (NAP1), restriction endonuclease analysis (REA) type BI, and PCR ribotype 027, collectively referred to as NAP1/BI/027.[5,18]

It should be noted that prior to the realization that *C. difficile* was the etiologic agent for the majority of cases of PMC, other infectious agents had been implicated as causative agents of PMC and AAD (reviewed by Gorkiewicz[19]). *Staphylococcus aureus* was one of the first bacteria associated with pseudomembranous enteritis following the administration of antibiotics.[20,21] Experimental evidence was provided by studies that demonstrated that antibiotic-treated chinchillas would develop enterocolitis after infection with *S. aureus.*[22] Although *C. difficile* is responsible for most cases of severe antibiotic-associated colitis, enterotoxigenic *S. aureus* strains can still be isolated from *C. difficile*–negative cases.[23] Enterotoxigenic *Clostridium perfringens* strains have also been isolated from patients with AAD.[24] Another form of severe antibiotic-associated colitis (associated with hemorrhage, but not pseudomembrane formation) appears to be due to infection with *Klebsiella oxytoca.*[25] These findings indicate that a proportion of cases of antibiotic-associated colitis and AAD that are due to infection are not secondary to *C. difficile,* but this is only in the minority of cases. Additionally, it needs to be restated that infection (including infection with *C. difficile*) accounts only for about a quarter of cases of AAD. In noninfectious cases of AAD, disruption of the normal composition and function of the indigenous gut microbiota alone, as detailed in the following section, is thought to be the underlying cause.[26]

PATHOGENESIS

The Indigenous Gut Microbiota and Antibiotics

The pathogenesis of antibiotic-associated colitis and AAD, whether or not they are associated with *C. difficile* infection, is thought to center on the disruption of the normal, indigenous gut microbiota by antimicrobial drug administration. It is important to note that the concept of the indigenous gut microbiota playing a central role in human health has existed for at least 100 years. Recent scientific and technologic advances have permitted the study of the role that the gut microbiome plays in human health and disease,[27,28] including the development of antibiotic-associated colitis and AAD.

The indigenous gut microbiota are thought to play a number of critical roles in host homeostasis and for the purposes of CDI, the function of importance has been called "colonization resistance."[29] Colonization resistance refers to the ability of the normal gut microbial community to resist the ingrowth of pathogenic microbes. A number of different mechanisms are speculated to be important for maintaining colonization resistance, including competition for nutrients, occupancy of ecologic and physical niches, production of antimicrobial products, and signaling through the host immune system (Fig. 243.1).[30] Regardless of the specific mechanisms, therapeutic antibiotic administration can profoundly disrupt the indigenous gut microbiota community and therefore disrupt colonization resistance. Studies in human and animal systems indicate that antibiotics can have long-lasting effects on the gut microbiome structure, and thus alter gastrointestinal function.[31–34] With regard to *C. difficile* the altered gut microbiome structure and function can directly influence the biology of the pathogen in terms of growth and pathogenesis. In the following sections, as specific topics in the pathogenesis and virulence of *C. difficile* are discussed, potential influences of the indigenous microbiota are highlighted.

Sporulation and Germination in *Clostridioides difficile*

As with many anaerobic bacteria, during periods of environmental stress vegetative *C. difficile* bacteria initiate the sporulation program, which results in the production of the spore form of the organism.[35,36] This physically robust form is stable to oxygen stress, temperature extremes, and desiccation. Within the hospital environment, this includes resistance to the effects of alcohol-based hand sanitizers.[37] The spore form thus represents a reservoir for the organism, which can persist in the environment.

Following ingestion of spores, germination is apparently triggered by the environment that exists within the gastrointestinal tract.[38] It has been shown that primary bile acids, including taurocholic acid, can serve as potent germinants in vitro, with glycine functioning as a cogerminant.[39–41] Interestingly, members of the indigenous microbiota efficiently metabolize primary bile acids through the process of deconjugation and dehydroxylation to produce secondary bile acids,[42–44] some of which have been shown to be inhibitory to *C. difficile.*[40,41,45] Therefore one mechanism by which the disruption of the indigenous microbiome by antibiotics can lead to CDI is through alteration of in vivo bile acid metabolism.[30,46] This leads to a situation that favors germination of ingested spores and the subsequent vegetative outgrowth of the pathogen. The key role of spore germination in the pathogenesis of *C. difficile* may serve as an attractive therapeutic target, as has been recently demonstrated with the use of a synthetic bile salt analogue that inhibits germination and has a protective effect in animal studies.[47] Similarly, restoration of bile salt metabolism by administration of a bacterium with 7-α-dehydroxylase activity could restore colonization resistance against *C. difficile.*[48]

Toxin Production

The histopathologic hallmark of *C. difficile* infection is damage to the mucosal epithelium with generation of an acute, neutrophil-predominant inflammatory response with the formation of a pseudomembrane consisting of sloughed epithelial cells, inflammatory cells, and a fibrinous exudate (Fig. 243.2). Damage to the epithelium is caused by the most well-characterized *C. difficile* virulence factor, the large glucosyltransferase toxins TcdA and TcdB.[49–52] Members of the large clostridial toxin family, TcdA and TcdB are produced within the gastrointestinal lumen during the stationary phase of vegetative growth of *C. difficile.* Following binding to a number of potential cell surface receptors,[53–56] these toxins are taken up by the cells of the mucosal epithelium through the process of receptor-mediated endocytosis.[57,58] Within the endosome the toxin undergoes autocleavage to release the catalytic subunit, which is transferred to the host cell cytoplasm.[59] Subsequently, the active subunit glycosylates host cell guanosine triphosphatases belonging to the Rho family of cytoskeletal regulatory proteins.[60,61] Intracellular activation of Rho guanosine triphosphatases leads to disruption of the actin cytoskeleton, ultimately leading to apoptotic and necrotic cell death. In cultured epithelial cells, cytoskeletal disruption results in cell rounding and cytotoxicity. In vivo these effects are manifested by loss of cell-cell tight junctions with subsequent increase in epithelial permeability.[62,63] TcdA and TcdB also induce the secretion of cytokines in host cells, including interleukin-8, which likely leads to the acute neutrophilic inflammatory infiltrate that characterizes *C. difficile* infection.[64] Recent data suggest that these two related toxins cause cell death and tissue damage via distinct pathways.[65,66]

Other Virulence Factors

Some strains of *C. difficile,* including the NAP1/BI/027 strains that are responsible for the recent global outbreaks of CDI, secrete a toxin that is a member of the clostridial binary toxins exemplified by the iota toxin from *Clostridium perfringens.*[67] *C. difficile* binary toxin (CDT) is

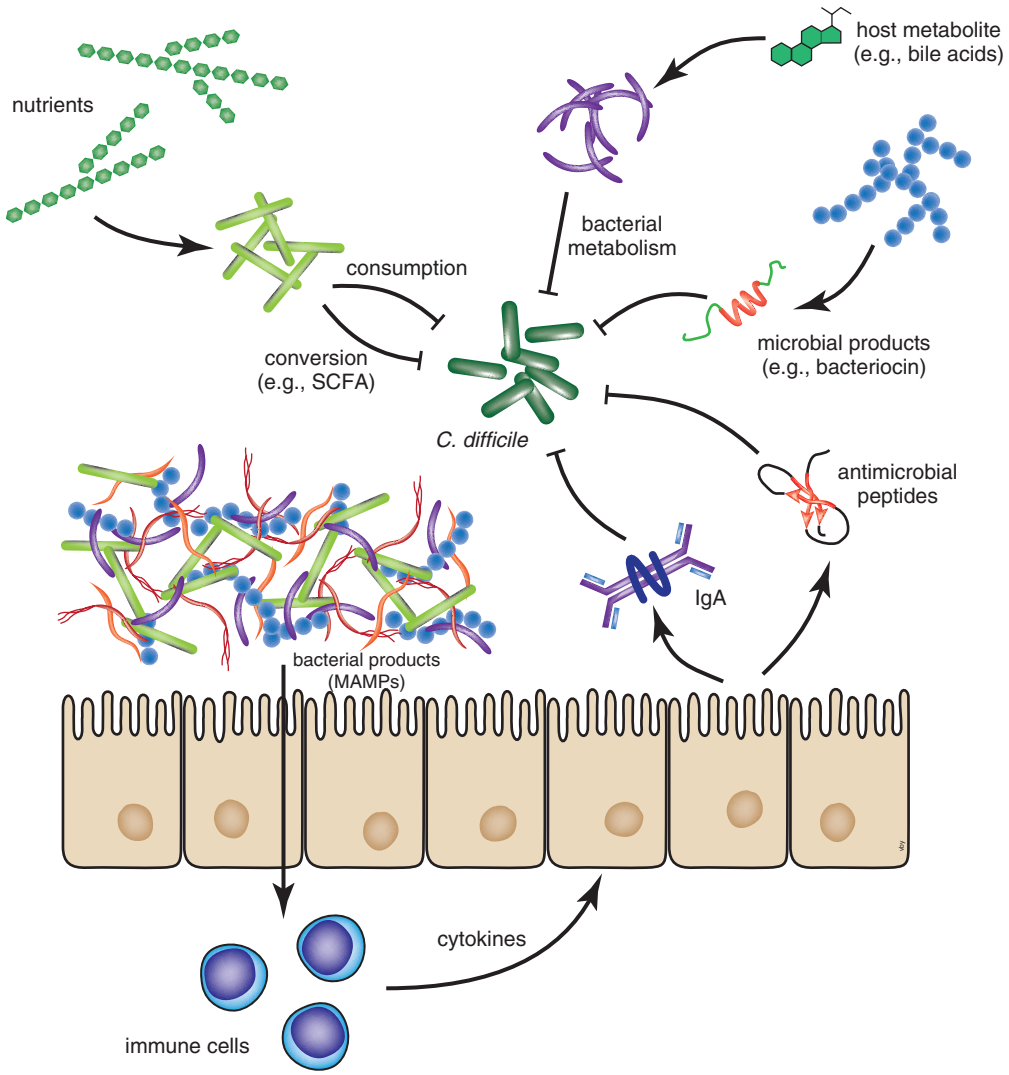

FIG. 243.1 Mechanisms by which the indigenous microbiota can mediate colonization resistance against *Clostridioides difficile*. *IgA,* Immunoglobulin A; *MAMPs,* microbial-associated molecular patterns; *SCFA,* short-chain fatty acid. *(From Britton RA, Young VB. Interaction between the intestinal microbiota and host in* Clostridium difficile *colonization resistance.* Trends Microbiol. *2012;20:313–319.)*

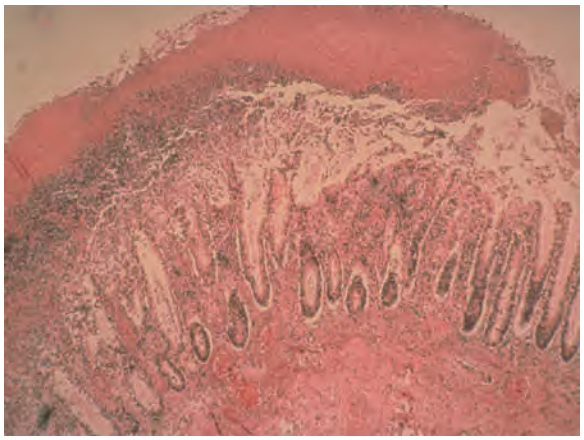

FIG. 243.2 Histopathology of pseudomembranous colitis. There is significant polymorphonuclear infiltrate, a fibrinous exudate, and epithelial damage.

a two-part toxin that is an ADP-ribosyltransferase specific for actin monomers, thus disrupting the actin cytoskeleton.[67] CDT is produced by only a small fraction of *C. difficile* strains, and thus its role in pathogenesis is unclear.[68] Although some reports suggest that infection with a CDT-producing *C. difficile* strain results in more severe clinical disease, this is not a universal finding and the actual significance of infection with CDT-producing strains is not clearly defined.[69–72]

In addition to the three toxins, multiple other potential virulence factors for *C. difficile* have been studied, including surface layer proteins, surface polysaccharides, flagella, and various adhesins.[73] To date, the specific roles of any of these factors in *C. difficile* virulence have not been delineated, but ongoing work on these factors may lead to new strategies to prevent and treat CDI.

Host Response to *Clostridioides difficile* Infection

Patients infected with *C. difficile* can mount adaptive immune responses to the toxins TcdA and TcdB.[74] The level of antibody response to the *C. difficile* toxins is inversely correlated with the relative risk of developing recurrent disease.[75] This finding served as the basis for the development of toxin-specific monoclonal antibodies for the prevention of CDI recurrence.[76] Additionally, because the pathogenesis of *C. difficile* is tightly linked to pathogen expression of TcdA and TcdB, an alternative approach to immunotherapy in the form of toxin-based vaccines is being explored.[77]

Additional work has been done examining the role of innate immune responses in the pathogenesis of CDI.[78] In animal models, mice that are deficient in Toll-like receptor signaling, including MyD88 knockout mice, were shown to be more susceptible to experimental CDI.[79,80] Similarly, animals deficient in signaling through the intracellular

pattern-recognition pathway involving Nod1 are more susceptible to *C. difficile* infection.[81] It appears that signaling through the innate immune system early during infection is important in host defense against *C. difficile*. Increased interleukin-23 signaling is encountered in humans with acute CDI, and this finding is replicated in murine models of infection.[82] Protection against acute disease apparently reflects the balance of several innate immune responses to *C. difficile* infection. Innate lymphoid cells (ILCs) of the ILC-1 type and to a lesser extent of the ILC-3 type are required for recovery from acute disease in a murine model.[83] Different studies demonstrated that eosinophils, stimulated by the microbiota, also provide protection in murine models of acute CDI.[84] Interestingly, these same investigators demonstrated that expression of binary toxin may increase virulence of *C. difficile* by suppressing this eosinophilic response.[85] These studies illustrate the complex system of interactions among the microbiota, host responses, and pathogen in the pathogenesis of CDI. Importantly, this may lead to novel treatment strategies for CDI, particularly severe acute disease.

Pathogenesis of Recurrent *Clostridioides difficile* Infection

While the majority of patients with symptomatic CDI respond to antibiotic therapy directed against the pathogen, up to 25% of patients will have recurrent symptoms following the discontinuation of *C. difficile* therapy for an initial episode of disease.[86,87] The risk of recurrence increases with each episode, resulting in significant morbidity and mortality in this population of patients. A number of factors have been investigated with regard to the pathogenesis of recurrent infection. As noted previously, the adaptive host response may play a role in determining the risk of recurrent disease.[75]

Several studies have indicated that pathogen characteristics may contribute to the risk of recurrence. Infection with the epidemic NAP1/BI/027 *C. difficile* strain is associated with an increased risk of recurrence.[88] The reasons for this are unclear, but may have to do with the intrinsic antibiotic resistance, the dynamics of spore biology of the strain, or the presence of an additional toxin, binary toxin.

Because alteration of the indigenous gut microbiota by antibiotics is a prerequisite for the majority of cases of CDI, the role of the gut microbiome in recurrence has been examined. It has been shown that patients with recurrent CDI exhibit decreased microbial diversity of their indigenous gut microbes.[89] Presumably, if the gut microbiota is unable to return to its baseline state, the patient is at increased risk of reinfection or regrowth of residual *C. difficile* following therapy. This has led to the exploration of restoring microbiome diversity through the administration of probiotic bacteria or the transplantation of feces from normal donors (see "Treatment" later).

Altered Virulence in Specific *Clostridioides difficile* Lineages

The increase in the incidence and apparent severity of CDI over the past 15 years has been associated with the widespread appearance of the NAP1/BI/027 strain of *C. difficile*. This has prompted a number of investigators to determine if this particular strain of *C. difficile* is actually associated with worse outcomes in infected patients and whether or not this strain has specific virulence determinants not widespread in other strains of the pathogen.

Several factors are characteristic of NAP1/BI/027 strains. These strains typically are resistant to newer fluoroquinolone antibiotics.[5] Furthermore they possess the binary toxin CDT, and also harbor a characteristic mutation in the anti-sigma factor *tcdC,* which is theorized to play a role in the regulation of toxins TcdA and TcdB.[5] This mutation in *tcdC* introduces a frameshift that results in a truncated, nonfunctional protein.[90] Because studies have demonstrated that these epidemic strains can produce increased levels of toxin in vitro, it has been proposed that this characteristic mutation is responsible for this phenotype, although this has been questioned by reconstitution of *tcdC* that showed no effect on toxin production.[80] There is in vitro evidence that the TcdC regulator can inhibit in vitro expression of the large *C. difficile* toxins,[90] but the presence of alterations within this regulatory gene does not directly correlate with increased production of toxins within clinical isolates.[91] One group demonstrated that complementation of the *tcdC* mutation in *trans* will decrease the amount of toxin produced by a NAP1/BI/027 clinical isolate and reduce the virulence of this strain in the hamster model of infection.[92] However, a second group that utilized a genetic system that allowed correction of the *tcdC* mutation directly on the chromosome failed to show an association between *tcdC* genotype and the level of toxin production.[93]

Additional in vitro studies suggested that NAP1/BI/027 strains had an increased ability to sporulate,[94] which could contribute to the ease with which these strains can spread within the health care environment. However, this observation has also been disputed by measuring sporulation in vitro in multiple *C. difficile* strains, including multiple NAP1/BI/027 strains that did not reveal increased sporulation of these epidemic strains.[95] Perhaps most important to the enhanced virulence of these strains is the presence of a third toxin, binary toxin CDT, an ADP-ribosylating toxin that has been shown to enhance the lethality of toxin A in the hamster model and to cause a modest degree of hamster mortality in the absence of toxins A and B.[96] It is interesting to note that this constellation of characteristics has also arisen within an unrelated epidemic strain of polymerase chain reaction (PCR) ribotype 078 that was identified in Europe (and that arose in swine)[97] and together with ribotype 027 was found to exhibit increased 14-day mortality in CDI patients.[98]

Despite this evidence, there is still debate over the relative importance of these genetic and phenotypic characteristics of the epidemic *C. difficile* strains in the pathogenesis of the organism.[99] In spite of these conflicting data, it is likely that our understanding of the basic pathogenesis of CDI will result in the development of more effective strategies for prevention and treatment of this infection.

EPIDEMIOLOGY

C. difficile is a ubiquitous organism, able to survive for long periods in the environment through sporulation. al Saif and Brazier showed the widespread presence of *C. difficile* in environmental sites such as surface water, drinking water, swimming pools, and soil, and in a wide variety of animals, including dogs, cats, horses, sheep, and pigs.[100] In addition, some foods have been shown to be contaminated at a low level with *C. difficile* spores that are of the same ribotype that cause clinical disease. This includes beef, pork, turkey, and a variety of vegetables, but there has been no evidence of foodborne transmission to date. It is highly likely that humans ingest *C. difficile* spores frequently, but remain asymptomatic (and uncolonized) as a result of the colonization resistance of an intact gut microbiota.

Health Care–Associated *Clostridioides difficile* Infection

It has been known since 1979 that the hospital environment is contaminated with *C. difficile* spores.[101] In addition, *C. difficile* contamination of the hands of hospital personnel and of the home environment of patients, and spore persistence for up to 20 weeks in the environment, have been known since 1981.[102] This environmental contamination is presumed to be the result of the repeated CDI diarrheal episodes of hospitalized patients and resistance of spores to killing by environmental cleaners and disinfectants other than bleach. In 1986, the first prospective case-control study of CDI was published and showed 87% of 149 cases to be health care associated (and hospital associated).[103] In addition, *C. difficile* was found asymptomatically in the stool of 21% of control patients. Both the specific use of clindamycin and use of multiple antibiotics for the treatment of infection were identified as risk factors. McFarland and colleagues[104] in a landmark paper used rectal swab specimens to identify hospital acquisition of *C. difficile* by 21% of sampled patients, 63% of whom were asymptomatic. Johnson and coworkers[105] also used weekly rectal swab cultures to document health care (in this case hospital) acquisition of *C. difficile* by 21% of patients; in this study, 82% of patients were asymptomatic. The use of REA typing identified 18 unique REA types of *C. difficile*, but symptomatic disease (CDI) was caused only by two closely related REA types, B1 and B2, which also caused asymptomatic colonization in some patients, but CDI in others.

It has also been shown that admission of asymptomatic patients carrying *C. difficile* in their stool to a hospital ward preceded the

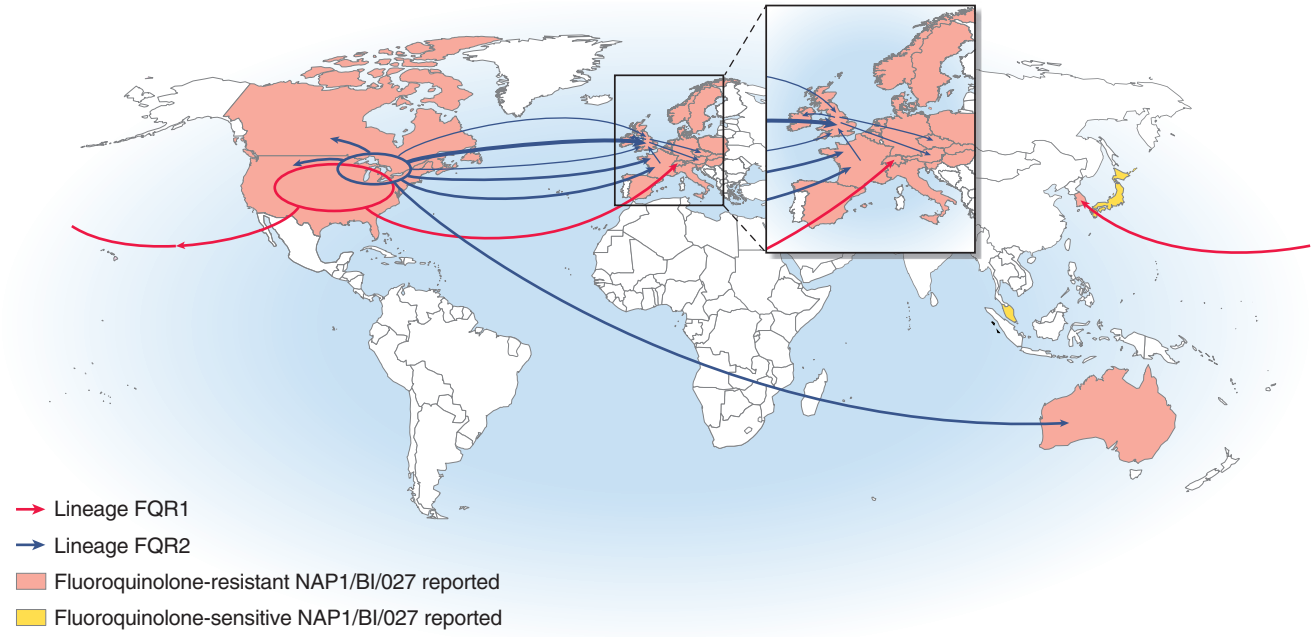

FIG. 243.3 Inferred global spread of the NAP1/BI/027 strain of *Clostridioides difficile*. *(From He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated* Clostridium difficile. Nat Genet. *2013;45:109–113.)*

acquisition of that specific strain of *C. difficile* by other patients on the same ward in 85% of acquisitions.[106] Although asymptomatic patients colonized with *C. difficile* may be a source of transmission to other patients, contrary to intuitive thinking, they have a significantly lower risk of developing CDI themselves compared to uncolonized patients on the same hospital wards at the same time.[107] It is presumed that these asymptomatic colonized patients are protected from CDI either because they harbor nontoxigenic strains of *C. difficile* (which lack toxin genes and do not cause symptoms) or they are protected from the effect of *C. difficile* toxins through adaptive immunity (most likely an anamnestic antitoxin antibody response) if they are colonized by toxigenic strains of *C. difficile*.

The health care epidemiology of CDI changed markedly in the early years of this century when multiple hospitals, first in the United States and then in Canada, reported widespread outbreaks of CDI of particularly high severity, causing high mortality and requiring increased use of colectomy to treat patients refractory to medical management.[5,18,108] These outbreaks were caused by a specific group of *C. difficile* strains designated NAP1/BI/027 that rapidly spread to the United Kingdom and European Union countries. Although unknown at the time, there were two distinct lineages of NAP1/BI/027, designated FQR1 and FQR2, that acquired fluoroquinolone resistance (FQR) mutations and a highly related conjugative transposon.[109] The FQR1 lineage almost certainly originated in the United States and was widely disseminated there, with spread to Asia and Switzerland (Fig. 243.3). The FQR2 lineage was found in both Montreal, Canada and multiple US sites and spread more widely than FQR1 to the United Kingdom and Europe. Health care–associated CDI incidence rose to as high as 22.5 CDI cases per 1000 discharges in the Montreal NAP1/BI/027 outbreak, with 30-day attributable mortality of 6.9%.[18] More recent reports suggest that the current hospital incidence in the United States is in the range of 5 to 10 CDI cases per 10,000 days of care.[110]

Community-Associated *Clostridioides difficile* Infection

There has never been a question that CDI occurs in the community setting, but whether the incidence of community-associated CDI versus community-onset CDI is increasing is an active epidemiologic question that is likely being affected by shorter inpatient stays and increased health care delivery in the outpatient setting. In particular, the use of antibiotics in the outpatient setting, calculated to amount to 258 million courses in 2010,[111] is likely to create enormous pressure for the development of community-associated CDI. Hirschhorn and colleagues[112] found the rate of community-associated CDI to be 7.7 cases/year per 100,000 population in a health plan database in Boston published in 1994. No antibiotic exposure was found in a surprising 35% of patients in the previous 42 days. More recently the US Centers for Disease Control and Prevention (CDC) has shown that community-associated CDI rates vary by geographic area and that independent predictors of higher community-associated CDI incidence are older age, white race, female gender, and use of nucleic acid amplification testing (NAAT). After adjusting for these predictors, community-associated CDI rates ranged from 30.7 to 41.3 per 100,000 population.[113] Conflicting community rates have been found in other large databases. One of these, the UK General Practice Research Database, found that CDI increased from less than 1 case per 100,000 in 1994 to 22 cases per 100,000 in 2004. Only 39% of these patients had prior antibiotic exposure documented within 90 days prior to CDI.[113] A reanalysis of the same data using a case definition of community-associated CDI as treatment with oral vancomycin found an association with prior antibiotic exposure in 55% of patients.[114] Using a more definitive methodology, a UK study of positive stool cultures from community-onset diarrhea patients showed 2.1% of stools positive for *C. difficile* toxin by cell cytotoxin assay in urban and rural patient cohorts, corresponding to rates of 29.5 and 20.2 cases per 100,000 population, respectively, much closer to more recent US rates.[115] Only 52% of these patients had antibiotic exposure in the previous 4 weeks.

A more recent report of community CDI epidemiology conducted by the CDC through its Emerging Infections Program has clarified a number of aspects of CDI in the community and health care settings.[110] In contrast to the 1986 report of 87% hospital-onset CDI, the more recent population-based report from the CDC of 10,342 CDI cases in 111 acute-care hospitals and 310 nursing homes revealed only 25% hospital-onset CDI, a similar proportion with nursing home onset, and 45% with community-onset CDI (Fig. 243.4). Among all CDI cases, 94% had either inpatient or outpatient health care exposure within the previous 12 weeks, leaving only 6% of all CDI cases with no prior health care exposure.

In a related study, of 989 community-associated CDI patients, only 18% had no outpatient health care exposures, 41% had low-level, and 41% had high-level outpatient exposures.[116] Of these, 358 (36%) did not receive antibiotics, but the incidence of antibiotic exposure was

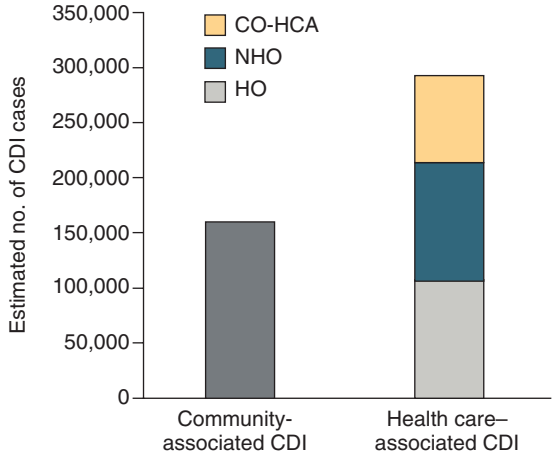

FIG. 243.4 Estimated burden of *Clostridioides difficile* infection *(CDI)* in the United States, 2011. Cases are divided into community-associated versus health care–associated cases. Of the community-associated cases, 82% were estimated to be associated with outpatient health care exposure. The health care–associated cases are divided into community-onset health care–associated cases *(CO-HCA),* hospital onset cases *(HO),* and nursing home–onset cases *(NHO). (From Lessa FC, Mu Y, Bamberg WM, et al. Burden of* Clostridium difficile *infection in the United States.* N Engl J Med. *2015;372:825–834.)*

significantly higher in the groups with health care exposure than in those without ($P < .01$), an indication that health care exposure predisposes to CDI via exposure to antibiotics. The frequency of exposure to proton pump inhibitors (PPIs) was not significantly higher in patients without antibiotic exposure than in patients with antibiotic exposure, suggesting that PPI use is not an independent or surrogate (for antibiotic use) risk factor for community-associated CDI. CDI patients with low-level health care exposures were more likely to be exposed to infants <1 year of age (odds ratio, 2.1; 95% confidence interval, 1.1–4.5) and household members with CDI (odds ratio, 6.9; 95% confidence interval, 5.6–56.5) compared to patients with high-level exposures, suggesting that these are *C. difficile* exposure sources. There was no association between food or animal exposure and level of health care exposure, leaving the source for acquisition of *C. difficile* undetermined for most patients.

Molecular Epidemiology of *Clostridioides difficile* Infection and Importance of Strain Type

The lack of a universally sensitive and reproducible typing system has slowed progress in the molecular epidemiology of CDI, but PCR ribotyping, pulsed-field gel electrophoresis, and REA are the most frequent typing systems in use. REA was used to document the surprising finding that approximately 50% of isolates from second episodes of CDI were different from the original isolate, suggesting an environmental source for recurrent CDI at least half of the time[117]; this observation has shifted recently toward more frequent identification of the same strain as the cause of recurrence (>80% of instances).[118] REA was also used to document the wide diversity of *C. difficile* strains in one hospital (55 different types) during an endemic period.[119] The REA J group found in multiple US hospitals in the 1990s was shown by PCR ribotyping in the United Kingdom to be ribotype 001.[120,121] These J/001 strains were clindamycin resistant, and increased CDI incidence was associated with clindamycin exposure in the patients. Molecular typing was instrumental in identifying the current epidemic *C. difficile* toxinotype III strains both by pulsed-field gel electrophoresis (type NAP1) and by REA (group BI) in hospitals in the United States and Canada, and confirmed in the United Kingdom and Europe as PCR ribotype 027.[5,18] These strains are highly resistant to fluoroquinolone antibiotics and are associated with fluoroquinolone use as a risk factor.[18] Typing using whole-genome sequencing suggests that there is a very diverse population of *C. difficile* strains causing CDI in hospitals.[122]

TABLE 243.1 Risks for Development of *Clostridioides difficile* Infection

Any antibiotic versus no antibiotic:
Number of antibiotics (risk increases with number)
Days of antibiotics (increased risk with increased days)
Type of antibiotic:
Highest risk: clindamycin, fluoroquinolones, cephalosporins of second generation and higher
Moderate risk: penicillins, macrolides, penicillin β-lactamase inhibitors, carbapenems, vancomycin, metronidazole
Lower risk: aminoglycosides, tetracyclines, trimethoprim, sulfonamides, rifampin
Proton pump inhibitors and histamine type 2 blockers
Patient age (increased risk with increased age of the patient)
Prior hospitalization
Severity of underlying illness
Abdominal surgery
Nasogastric tube
Duration of hospitalization
Long-term care residency

Clostridioides difficile Infection in Children

Hall and O'Toole first described *C. difficile* in 1935 as a colonizing organism in newborn infants.[123] Ironically this continues to be a contentious diagnostic issue in children up to the age of 1 to 2 years because the frequent asymptomatic colonization of these young children confounds the diagnosis of diarrhea; if tested they frequently will be positive for toxigenic strains of *C. difficile,* but the frequency of stool positivity for *C. difficile* in these children with diarrhea is no higher than that of children of the same age without diarrhea.[124] It is not known how very young children can tolerate colonization by toxigenic *C. difficile* without developing diarrhea or other symptoms. Data from newborn rabbits suggests that there may be insufficient toxin A receptors in these young animals that allows them to tolerate toxin, but similar observations in humans have not been made.[125] When children are sampled on a monthly basis over the first year of life, all are found to at some time be colonized with *C. difficile,* both toxigenic and nontoxigenic strains, and they are noted to frequently change strains over time.[126] Because of the high rate of colonization in children under the age of 1 to 2 years, it is not recommended that they be tested for CDI.[125] Beyond the age of 3 years the colonization rate of children resembles that of adults (<3%) and risk factors for CDI are also similar to those in adults, but CDI rates are generally much lower in children than in adults, especially adults over age 65 years.[124,127] Health care–associated CDI in children is associated with prolonged hospitalization and increased mortality, whereas community-associated CDI is not.[128]

Risk Factors

Risk factors have been identified for CDI, for recurrent CDI, for CDI severity, and for CDI caused by the epidemic NAP1/BI/027 strain.

Risk for *Clostridioides difficile* Infection

The overall risk for developing CDI is dominated by antimicrobial exposure, including duration, number, and class of antimicrobials (Table 243.1).[129] Risk is highest during therapy and in the first month after cessation of antimicrobial therapy and decreases between 1 and 3 months posttherapy.[130] In addition, stomach acid–inhibiting agents such as PPIs and histamine type 2 antagonists have also been associated with increased risk for CDI, although some studies fail to make this association.[131] It is likely that the combination of an antimicrobial and a PPI increases the risk for CDI.[132] Patient factors are also important CDI risks, with advanced age, immunosuppression, prior hospitalization, and severity of underlying illness contributing to increased risk.[133] Absence of antitoxin antibodies and waning antibody levels with increasing age are likely additional risks for CDI. A meta-analysis and strain risk comparisons have identified fluoroquinolone exposure and age over 65 years as risks for CDI caused by NAP1/BI/027.[134,135]

Risk for Recurrent *Clostridioides difficile* Infection

Risk factors for recurrent CDI are similar to those for an initial episode but are particularly predictable because one antecedent episode of CDI increases risk with each subsequent recurrence (Table 243.2). Additional antimicrobial treatment either during or after the initial CDI episode markedly increases risk of recurrence, and as with primary CDI, the most elderly patients and those with the most severe underlying disease are at highest risk of recurrence.[136–138]

Risk for Severe *Clostridioides difficile* Infection

There has been an ongoing search for a clinical prediction rule or score that would predict at the time of diagnosis which patients are destined for a severe CDI outcome, usually defined as death, need for intensive care unit admission, megacolon, perforation, shock, or requiring colectomy for treatment. A number of patient predictors, such as a white blood cell (WBC) count >15,000 or 20,000/μL and a rise in creatinine of >1.5 times baseline (or a serum creatinine ≥1.5 mg/dL), have correlated with more severe disease.[4,139,140] Low serum albumin and elevated WBC count have been found to predict CDI severity independent of infecting strain type, but higher 14-day mortality has been observed with infection caused by two strains of *C. difficile*—NAP7-8/BK/078 and NAP1/BI/027—as well as with the presence of specific patient biomarkers: elevated WBC and C-reactive protein, and low serum albumin.[99,141] Additionally, infection with the NAP1/BI/027 strain of *C. difficile* has resulted in lower cure rates and higher recurrence rates compared to other *C. difficile* strains when treated with either fidaxomicin or vancomycin.[142] Although several studies have demonstrated the association between infection with NAP1/BI/027 strains and increased disease severity, this has not been a universal finding.[99]

Infection Control and Prevention

Infection control practices for CDI are of two primary types: prevention of *C. difficile* transmission to the patient, and reduction of the likelihood of CDI developing in the event that a patient encounters *C. difficile* or its spores (Fig. 243.5). Current guidelines for prevention of CDI in hospitals focus on patients with symptomatic infection and emphasize the importance of optimizing basic practices before considering addition of special approaches with less supporting evidence or greater potential for undesirable outcomes.[143] For prevention of transmission, the basic practices include contact precautions (i.e., personnel wear gloves and gowns for any contact with the patient or environment and patients are placed in a single-patient room with dedicated noncritical patient care equipment) and efforts to ensure adequate cleaning and disinfection of equipment and the environment.[143] Gloves are recommended because alcohol hand sanitizers are ineffective in reducing spores of *C. difficile* on hands.[37,144,145] In addition, there is evidence that requiring health care workers to wear gloves when handling the body substances of patients can reduce the incidence of CDI.[146] Sporicidal disinfectants (e.g., bleach, peracetic acid–based products) are recommended, particularly in outbreak or hyperendemic settings, based upon multiple studies reporting reductions in CDI when bleach was substituted for nonsporicidal disinfectants.[147] The recommendation for dedicated noncritical patient care equipment (e.g., thermometers, stethoscopes) for CDI cases is based on evidence that such equipment may frequently become contaminated with spores.[148] Moreover, replacement of electronic thermometer use (the handles become contaminated with spores of *C. difficile*) by disposable thermometers has been associated with significant reductions in CDI rates.[149]

If basic practices are unsuccessful in controlling CDI, health care facilities may consider adding special approaches that address plausible sources of transmission. Patients with acute CDI shed large numbers of spores,[150] and delays in diagnosis are not uncommon.[151] In addition, many patients with CDI continue shedding spores as asymptomatic carriers for several weeks after treatment[152] (see Fig. 243.5). Based on these findings, many hospitals preemptively isolate patients with orders for CDI testing, or extend the duration of contact precautions to hospital discharge, or both. The potential benefit of expanded use of contact precautions must be balanced against the potential for adverse effects.[143] Given that up to 25% of initial CDI cases will develop a recurrence, extending the duration of contact precautions also ensures that many patients with recurrent CDI will be under contact precautions when their symptoms recur. Monitoring of environmental cleaning with feedback to cleaning personnel, daily disinfection of surfaces in CDI rooms, and adjunctive use of no-touch disinfection technologies (e.g., ultraviolet light room decontamination devices, hydrogen peroxide vapor) can reduce spore contamination in patient rooms, and have been associated with reductions in CDI in quasi-experimental studies.[147,153,154] However, in two recent multicenter randomized trials, interventions to improve cleaning and disinfection through monitoring and feedback or ultraviolet light room decontamination did not reduce

TABLE 243.2 Risk Factors for Development of Recurrent *Clostridioides difficile* Infection

Any prior episodes of *C. difficile* infection, particularly severe infection
Antibiotic use (concomitant or post–*C. difficile* infection treatment, or both)
Age ≥65 years
Prolonged or recent stay in health care facility
High severity of Horn index for underlying illness
Immunosuppression
Proton pump inhibitor use
Infection with NAP1/BI/027 strain
Absence of an antitoxin A antibody response

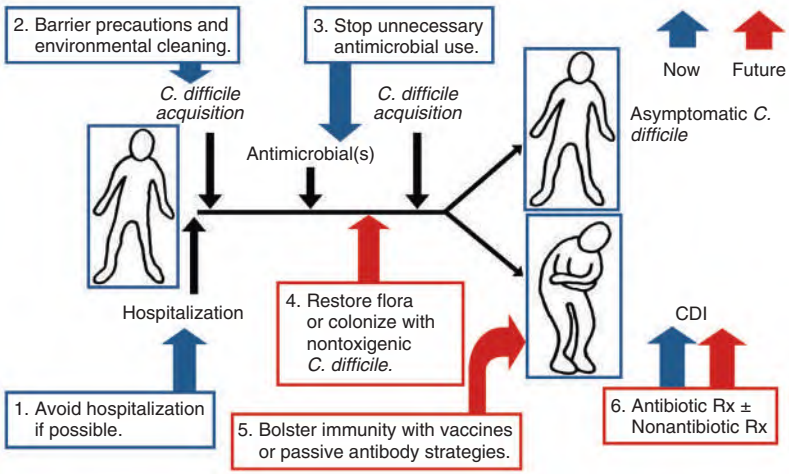

FIG. 243.5 Sites of attack for present and future prevention and management of *Clostridioides difficile* infection. *(From Gerding DN, Johnson S. Management of* Clostridium difficile *infection: thinking inside and outside the box.* Clin Infect Dis. *2010;51:1306–1313.)*

health care–associated CDI in comparison to standard cleaning with bleach.[155,156] In a secondary analysis of one of these trials, addition of ultraviolet light room decontamination to standard cleaning in isolation rooms was associated with a significant but modest reduction in hospital-wide incidence of CDI, but no reduction was seen when ultraviolet light was added to bleach disinfection.[157]

One limitation of current approaches for prevention of *C. difficile* transmission is that asymptomatic carriers of toxigenic *C. difficile* are not addressed. Two recent studies suggest that asymptomatic carriers may be an important source of transmission.[158,159] Moreover, in a controlled quasi-experimental study in a Canadian acute-care hospital, detecting and isolating asymptomatic carriers was associated with a significant decrease in the incidence of health care–associated CDI.[160] Given the difficulties involved in implementing screening programs for *C. difficile* carriage, additional high-quality studies are needed to evaluate the impact of routine screening and isolation. A more feasible approach might be to extend the duration of contact precautions after CDI symptoms resolve because patients with prior CDI are an easily identified subset of asymptomatic carriers (~20% of all carriers).[143,161]

Preventing patients from encountering and ingesting *C. difficile* while in the hospital is extremely difficult given the survival durability of spores and their resistance to commonly used hand sanitizers and disinfectants. Rendering the patient host less susceptible to disease if spores are ingested is an alternative method to prevent infection. The most effective method to reduce the risk for CDI is to reduce overuse of all antimicrobials or of specific high-risk agents, including cephalosporins, clindamycin, and fluoroquinolones. In a recent systematic review and meta-analysis, implementation of antimicrobial stewardship interventions was associated with a 32% overall reduction in the incidence of CDI.[162] Restriction of clindamycin or fluoroquinolones has been associated not only with significant reductions in CDI, but also with elimination of clindamycin- or fluoroquinolone-resistant epidemic strains causing outbreaks, including NAP1/BI/027 epidemic strains.[163,164] Current guidelines recommend that all health care facilities encourage appropriate use of antimicrobials as a basic CDI prevention practice, and consider more intensive stewardship interventions, including restriction of high-risk agents based on local CDI epidemiology.[143]

It is anticipated that a number of experimental measures will be effective in the future in preventing CDI, including *C. difficile* vaccines and biotherapeutics (use of live organisms to restore colonization prevention) given to patients who have taken antibiotics (see Fig. 243.5).[165] Both of these approaches are undergoing human clinical trials and are expected to become available for general use.[166–169] Recently, monoclonal antibodies directed against *C. difficile* toxins have become available as a potential approach to reduce the risk for recurrence of CDI in patients with primary or recurrent CDI.[76]

Use of probiotics (available as nutritional supplements) to prevent AAD and CDI has had a mixed experience. Efficacy in reducing AAD has been demonstrated in a number of studies, particularly in children; however, the wide diversity of organism content and dosage of available products has made it difficult to recommend specifically effective agents or combinations. Randomized trials of various probiotic products that have demonstrated benefit have either been underpowered or poorly designed, with rates of CDI in control groups so high that they challenge credibility.[170,171] *Saccharomyces boulardii* was shown to be marginally effective in preventing recurrent CDI when combined with high-dose (2 g/day) vancomycin but not when combined with lower vancomycin doses or with metronidazole, and was more effective in recurrent CDI than primary CDI, where it was ineffective in preventing infection and recurrent episodes.[172,173] The subject of preventive effectiveness of probiotics has undergone multiple meta-analyses, the most recent of which suggests that there may be a benefit in prevention of primary CDI in patients taking antibiotics.[174,175] However, the field needs rigorous, well-powered studies using well-defined products to provide an adequate scientific basis for a practice that is poorly documented.

CLINICAL AND PATHOLOGIC MANIFESTATIONS

Infection with a toxin-producing strain of *C. difficile* can be asymptomatic, especially in certain populations, including infants and patients in hospitals and chronic care facilities.[102,176,177] In patients who develop symptomatic disease during a course of therapeutic antibiotics, symptoms can occur concurrently with the initiation of antibiotics or even several weeks after the discontinuation of therapy.

Diarrhea due to CDI can range in severity from minimal, self-limited disease to profuse, with 20 or more episodes a day. About 20% to 25% of patients can be expected to resolve symptoms by stopping the offending antibiotics without using specific CDI treatment. Associated signs and symptoms can include fever, abdominal pain, and tenesmus.[178] Leukocytosis is a common feature of CDI, and a significant leukemoid reaction can be seen with peripheral WBC counts >25,000/mm^3 and exceeding 100,000/mm^3 in rare cases. Significant WBC count elevation can serve as a marker of severe disease, along with hypoalbuminemia and elevation of baseline serum creatinine levels.[179] Complications of the most severe forms of CDI include hypotension or shock, the development of toxic megacolon (suggested by radiographic evidence of acute dilatation of the colon to >6 cm), intestinal perforation, and acute peritonitis. Extraintestinal manifestations of CDI are surprisingly rare, including very infrequent evidence of bacteremia or peritonitis in the absence of perforation.

By endoscopic examination, the initial lesions of PMC initially appear as 1- to 2-mm whitish-yellow plaques that appear on a background of normal-appearing mucosa (Fig. 243.6).[178] As the disease progresses, these plaques coalesce and, as they become confluent, form the typical pseudomembrane covering the entire colon wall. Generally the entire colon is involved, but 10% of patients have rectal sparing. Although endoscopy is not part of the typical diagnostic strategy for diagnosis of CDI (see "Diagnosis" next), it can be useful when there is an acute abdomen or coexisting conditions such as inflammatory bowel disease.[180]

DIAGNOSIS

Diagnosis of CDI is based on the presence of clinical symptoms (usually defined as ≥3 watery, loose, or unformed stools within a period of 24 hours or less) coupled with a diagnostic test (usually of a stool specimen) that detects the presence of either the *C. difficile* organism or its toxin genes, or detection of *C. difficile* toxin using an enzyme immunoassay or cell cytotoxin assay. Diagnosis can also be made in patients with diarrheal symptoms by the use of lower gastrointestinal endoscopy to visualize pseudomembranes in the colon, but this method is used sparingly and is far more costly and less sensitive than a variety of stool diagnostics (Table 243.3). The presence of appropriate symptoms prior to stool testing is critical because *C. difficile* can be carried asymptomatically, especially by hospitalized patients.[181] Since the pathogen and its toxins can be readily detected with most of the available tests, detection of *C. difficile* in the absence of symptoms does not meet the criteria for a CDI diagnosis.

There is considerable controversy surrounding the most appropriate stool test to use for CDI diagnosis. The first test used, and one of the gold standards, was the cell cytotoxicity assay, which was developed contemporaneously with the discovery of *C. difficile* and its toxins as the cause of CDI.[182] The test is performed by taking dilutions of supernatants of stool, placing them on cell culture lines (a variety of cell types can be used) and incubating them for 24 to 48 hours and then observing microscopically for cell rounding, an indication of the presence of toxin B (and occasionally toxin A). Confirmation that cell rounding is due to *C. difficile* toxin is done by neutralizing the assay with *C. difficile* or *Clostridium sordellii* antitoxin (the latter of which cross reacts with *C. difficile* toxins).

Soon after the cell cytotoxicity assay was developed, a selective media containing cycloserine and cefoxitin for the culture of *C. difficile* from stool was described, paving the way for the second gold standard test, stool culture for toxigenic *C. difficile*.[183] The first enzyme immunoassay (EIA) for toxin A was described in 1983, followed by development of a monoclonal antibody for toxin A that set the standard for CDI testing for decades to come. Although not nearly as sensitive as the two gold standard tests, cell cytotoxicity assay and toxigenic culture (culture of *C. difficile* from stool and confirmation of toxin production in vitro), EIA was less labor intensive and provided more rapid turnaround of test results.[184,185]

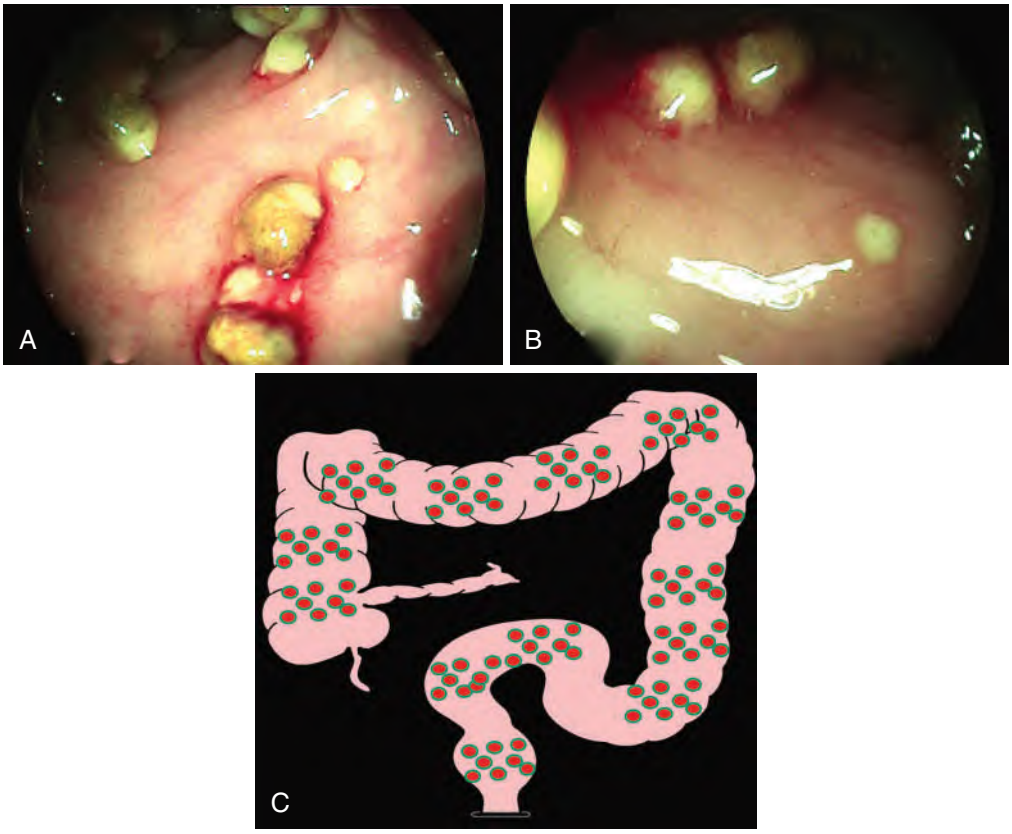

FIG. 243.6 Colonoscopic view of pseudomembranous colitis. (A and B) Discrete lesions surrounded by normal-appearing mucosa are seen early in disease. Hemorrhage is seen at the edges of some pseudomembranes (A) in this patient with underlying leukemia. (C) The anatomic location of the lesions.

TABLE 243.3 Endoscopic and Stool Diagnostic Tests for *Clostridioides difficile* and Its Toxins

TEST	SENSITIVITY (%)	SPECIFICITY (%)	COMMENT
Colon endoscopy	~50	100	Sensitivity and specificity are for detection of PMC
Cell cytotoxicity	77–86	97–99	The less sensitive of two gold standards compared with toxigenic culture
EIA for toxin A	67–92	93–99	Versus cell cytotoxicity
EIA for toxin B	60–89	93–99	Versus toxigenic culture
EIA for GDH	71–100	67–99	Compared with stool culture for *C. difficile*
Toxigenic culture for *C. difficile*	95–100	96–100	The more sensitive of two gold standards compared with cell cytotoxicity
Nucleic acid amplification test (PCR and LAMP)	88–100	88–97	Versus toxigenic culture Most sensitive rapid single test available but also expensive
Two-step GDH testing[a]	56–90	81–97	Discrepancy between GDH and toxin test is 13%–19%
Three-step GDH testing[b]	83–100	93–100	

EIA, Enzyme immunoassay; *GDH,* glutamate dehydrogenase; *LAMP,* loop-mediated isothermal amplification; *NAAT,* nucleic acid amplification test; *PCR,* polymerase chain reaction; *PMC,* pseudomembranous colitis.
[a]Two-step GDH testing: EIA for GDH and EIA for toxins A and B.
[b]Three-step GDH testing: EIA for GDH and EIA for toxins A and B, arbitrated by NAAT for discrepancies.

After decades of use, the toxin A EIA test became obsolete after it failed to detect increasingly frequent clinical outbreaks of CDI in multiple hospitals caused by *C. difficile* A−/B+ organisms that do not produce toxin A but cause CDI.[186] The toxin A EIA test was subsequently replaced with a variety of toxin A/B EIA tests utilizing a monoclonal antibody for detection of toxin A and polyclonal antibodies for detection of toxin B. These assays have been found to be relatively insensitive when compared with the cell cytotoxicity assay and toxigenic culture of *C. difficile* (see Table 243.3).[187,188] Not only are the EIA assays less sensitive than cell cytotoxicity assays, but cell cytotoxicity assays are less sensitive than toxigenic culture.[189,190]

A latex agglutination test developed in the 1980s reputedly for the detection of toxin A was subsequently found to detect another protein, glutamate dehydrogenase (GDH), which is not specific to *C. difficile* and is present in both toxigenic and nontoxigenic *C. difficile* isolates.[191] However, when GDH was converted from a latex agglutination to an EIA platform, the sensitivity was markedly improved, and the use of GDH testing (also known as common antigen) as part of a two-step or three-step test algorithm (GDH requires pairing with one or more tests for toxin to identify toxigenic *C. difficile*) has become one of the choices to replace toxin EIA alone with more sensitive tests. Utilizing the high negative predictive value of the GDH EIA test and the level of test positivity in the laboratory, 80% to 95% of specimens can be reported as *C. difficile* negative with rapid turnaround of results in the laboratory.[192] However, confirmation that a positive GDH specimen is due to a toxigenic strain requires doing a cell cytotoxin assay of the

stool to confirm presence of *C. difficile* toxin, and this adds 2 to 3 days to the test reporting. Because of the poor sensitivity of the EIA assays for toxins A and B, a number of two-step algorithms have been devised by the European Society for Clinical Microbiology and Infectious Diseases utilizing EIA testing for GDH and for toxins A and B.[193] To simplify the test process and improve the specificity of these two-step algorithms, manufacturers have designed tests that combine both EIA assays in a single test.[194] When results of GDH and toxin A/B EIA are discrepant (13%–19% of tests), the difference can be resolved by NAAT, which results in rapid turnaround of results (the so-called three-step algorithm) and is less expensive than testing all specimens with NAAT.

Improved CDI test sensitivity can also be obtained by using NAAT technologies alone, which are targeted to amplify conserved regions of either toxin A or toxin B genes using either PCR or loop-mediated isothermal amplification technology. NAAT detects the presence of a toxin gene in stool and thus is a test for detection of the presence of toxigenic *C. difficile,* similar to doing a toxigenic culture, but is not a test for toxin in the stool. GDH testing also detects the presence of *C. difficile,* but does not distinguish toxigenic from nontoxigenic strains. Use of PCR as a stand-alone test for CDI is increasing rapidly in laboratories in the United States and Europe and because of the increased sensitivity, apparent rates of CDI have increased by 50% or more in hospitals that have converted to its use. NAAT is the most sensitive rapid single test for *C. difficile* currently available and has sensitivity of 88% to 100% when compared to toxigenic culture (see Table 243.3).[190] Although NAAT is increasingly being employed by laboratories, because of high cost many laboratories are testing first with a GDH EIA or a GDH/toxinA/B EIA (two-step algorithm) and resolving discrepant GDH and toxin A/B EIA results with NAAT to reduce test costs (three-step algorithm) while retaining rapid test result reporting (see Table 243.3).

The increased rate of CDI diagnosis by NAAT has led some investigators to question whether NAAT is too sensitive as a stand-alone test.[195] The concern is that there is no test for toxin in the stool when NAAT is used alone, and that NAAT may be detecting *C. difficile*–colonized patients who have diarrhea for some other reason. Several studies have found that when NAAT is used alone, it detects more CDI cases than a three-step algorithm using GDH/toxinA/B EIA and cell culture cytotoxicity for discrepancies.[196,197] When patients who were diagnosed by NAAT alone ($N = 29$) were compared to patients diagnosed by both NAAT and the three-step algorithm ($N = 56$), it was found that patients diagnosed with NAAT alone had significantly lower rates of mortality, intensive care unit admission, readmission for CDI, and colectomy than patients diagnosed with the three-step algorithm ($P < .001$).[196] Similarly, in a recent larger study, 293 of 1416 patients were found to be NAAT positive (PCR+) but only 131 to be both PCR+ and toxin+ by EIA. The rates of complications (colectomy, megacolon, intensive care unit care) and CDI-related death at 30 days were significantly higher in the PCR+/toxin+ patients than in the PCR+/toxin− patients ($P < .001$).[197] The critical question is whether patients diagnosed by NAAT alone require treatment. They may have mild CDI that may or may not require treatment, or they may be asymptomatic carriers and require no treatment but should be isolated nonetheless because they may transmit *C. difficile,* particularly while having diarrhea. In the United Kingdom a two-step algorithm utilizing GDH EIA or NAAT for initial testing followed by a sensitive EIA for toxin A/B has been guideline recommended based on evidence of greater all-cause 30-day mortality and CDI complications when toxin is detected in stool compared to detection of only a toxigenic *C. difficile* organism.[198] The specific vulnerability of this algorithm is the reliance on an EIA test for detection of toxin in stool, a somewhat insensitive test in past experience. The 2017 Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) CDI Guidelines in the United States recommend that hospitals base the diagnostic test used on the presence of 3 or more diarrheal stools within 24 hours prior to testing, in which case either NAAT or a toxin-containing algorithm is recommended. If hospitals and laboratories do not agree to a diarrhea definition prior to testing, only an algorithm utilizing toxin testing is recommended, but not NAAT alone.[199]

The advent of more sensitive tests for CDI, such as NAAT or GDH algorithms, has made the use of repeated stool testing for diagnosis unnecessary.[200] A single test is sufficient to make or rule out the diagnosis of CDI, and tests should not be repeated for at least 7 days unless the clinical presentation changes. In addition, because these tests are so sensitive, they should not be used to evaluate response to therapy—the so-called test of cure—because the majority of successfully treated CDI patients will continue to have detectable *C. difficile* in stools posttreatment for 6 weeks or more.[152,201] Presence of continued *C. difficile* in stool is not an indication of failed therapy. Successful treatment is measured by the clinical resolution of symptoms, not microbiologic elimination of the pathogen.

TREATMENT

Before effective treatment for CDI was known, symptomatic patients were treated by stopping the offending antibiotic and giving fluid and electrolyte support if needed. About 20% to 25% of patients resolved their symptoms when this was done. When effective antimicrobial treatment was discovered, the practice of stopping the offending antibiotic and giving supportive treatment for 24 to 72 hours to see if the patient would respond before initiating treatment with vancomycin or metronidazole was continued.[202] This practice changed in the early years of the 21st century when previously unrecognized fulminant CDI with ileus, shock, and rapid progression to death occurred frequently and required rapid institution of specific treatment to save the life of the patient.[5,18,108]

Effective treatment of CDI with vancomycin was demonstrated as early as 1981; however, it was also shown that recurrences were frequent.[203] Because the cost of vancomycin was quite high at the time, alternative treatments were sought and a prospective randomized trial was conducted that showed vancomycin and metronidazole were comparable for CDI treatment and markedly reduced the cost of treatment.[202] Thus as early as 1983, two effective drugs for treatment of CDI were identified and used extensively with comparable results until the end of the 20th century, although metronidazole has never been approved for CDI treatment by the US Food and Drug Administration (FDA).[204] Vancomycin dosing for CDI was typically 500 mg 4 times a day initially, but to reduce cost a comparison study of 125 mg 4 times a day was conducted and the lower dose was found to be as effective as the higher dose.[205] There ensued considerable debate over the years as to whether vancomycin or metronidazole was the superior treatment agent for CDI despite small comparative trials that showed no difference. A randomized, blinded, stratified prospective trial published in 2007 showed metronidazole and vancomycin to be similar in treatment response for mild CDI, but that vancomycin was superior for severe CDI ($P < .02$).[206] This study confirmed the increasing clinical impression that metronidazole was not as effective in treating CDI as vancomycin. Despite numerous attempts, the reduced clinical effectiveness of metronidazole could not be attributed to *C. difficile* resistance to metronidazole. More recently, publication of two phase III randomized blinded clinical trials of the toxin-binding polymer tolevamer, which had two comparator treatment arms of vancomycin and metronidazole, also showed in a subanalysis of the largest such study to date that vancomycin was superior to metronidazole for treatment of all CDI patients using a multivariate analysis ($P = .034$). The 2017 IDSA/SHEA CDI Guidelines no longer recommend metronidazole as first-line therapy for CDI, replacing it with vancomycin or fidaxomicin.[199,207]

The importance of treating only symptomatic patients became apparent when it was discovered that asymptomatic patients colonized with *C. difficile* were commonly found in hospitals. They were thought at the time to be at risk of developing CDI, and a decolonizing treatment trial of asymptomatic patients was conducted comparing placebo versus vancomycin versus metronidazole.[208] In 9 of 10 patients treated with oral vancomycin, *C. difficile* organisms could not be detected during and immediately after treatment, compared with 3 of 10 patients treated with metronidazole ($P = 0.02$) and 2 of 10 patients given placebo ($P = 0.005$).[208] This initial effect could be explained because the mean fecal concentration of the unabsorbed vancomycin was over 1400 μg/g feces, whereas metronidazole, which is well absorbed in the absence of diarrhea, was not detectable in the feces of 9 of 10 patients. However, at the 2-month follow-up, 8 of 9 evaluable patients who received vancomycin remained colonized, 5 with new strains of *C. difficile,* compared to 2 of 10 placebo patients ($P < .005$) and 3 of 10 metronidazole patients (P

TABLE 243.4 Current Therapy for *Clostridioides difficile* Infection Based on Severity and Recurrence

CDI TYPE	ANTIBIOTIC	DOSE[a]	ALTERNATIVES
C. difficile (nonsevere)	Vancomycin, *or* Fidaxomicin	125 mg PO qid × 10 d 200 mg PO bid × 10 d	If vancomycin or fidaxomicin is not available or contraindicated, metronidazole 500 mg PO tid × 10 d
C. difficile (severe[b])	Vancomycin, *or* Fidaxomicin	125 mg PO qid × 10 d 200 mg PO bid × 10 d	None
C. difficile (severe complicated or fulminant)	Vancomycin + Metronidazole	500 mg PO qid × 10–14 d 500 mg IV q8h × 10–14 d	Tigecycline, 50 mg IV bid × 10–21 d in place of metronidazole Additional vancomycin via rectal retention enema, 500 mg in 100 mL normal saline q6h if complete ileus present Surgical colectomy or ileostomy
C. difficile (first recurrence)	Vancomycin, *or* Vancomycin taper and pulse, *or* Fidaxomicin	125 mg PO qid × 10 d if metronidazole was used for the initial episode 125 mg PO qid × 10 d, bid for 1 wk, qd for 1 wk, and then every 2 or 3 d for 2–8 wk if standard vancomycin was used for the initial episode 200 mg PO bid × 10 d if vancomycin was used for the initial episode	None
C. difficile (>one recurrence)	Vancomycin taper and pulse, *or* Vancomycin/rifaximin, *or* Fidaxomicin, *or* Fecal microbiota transplantation[c]	125 mg PO qid × 10 d, bid for 1 wk, qd for 1 wk, and then every 2 or 3 days for 2–8 wk Vancomycin 125 mg PO qid × 10 d followed by rifaximin 400 mg PO tid × 20 d 200 mg PO bid × 10 d	None

[a]All randomized trials have compared 10-day treatment courses, but some patients (particularly those treated with metronidazole) may have delayed response to treatment and clinicians should consider extending treatment duration to 14 days in those circumstances.
[b]The criteria proposed for defining severe or fulminant CDI are based on expert opinion. These may need to be reviewed in the future upon publication of prospectively validated severity scores for patients with CDI.
[c]The opinion of the panel is that appropriate antibiotic treatments for at least two recurrences (i.e., three CDI episodes) should be tried prior to offering fecal microbiota transplantation.

< .019). For asymptomatic *C. difficile* colonization, which poses little risk to the patient, vancomycin is effective as a temporary decolonizer, and metronidazole is ineffective, but colonization seems to be a transient phenomenon that resolves without any treatment and is only prolonged with vancomycin treatment, which presumably further disrupts the restoration of colonization resistance by the normal microbiota.[107] As such, routine treatment of asymptomatically colonized patients is not recommended.

Treatment recommendations for CDI have been developed for mild, moderate, severe, and severe complicated (also called fulminant) CDI, and for first and multiple recurrences of CDI. Recommendations adapted from the IDSA/SHEA Guidelines are shown in Table 243.4 together with alternative treatments that may also be effective.[140] For all listed treatments, the primary goal of successful treatment is the elimination of symptoms, and the secondary goal is prevention of recurrent CDI. Microbiologic persistence of *C. difficile* in stool occurs in the majority of patients following successful treatment, peaking at about 2 weeks posttreatment and persisting in about 5% for 6 months.[209] Repeat stool testing following treatment as a "test of cure" should *not* be done because it is not a predictor of either treatment success or failure, and will lead to unnecessary treatments, testing, and likely morbidity.

Mild-to-Moderate *Clostridioides difficile* Infection

Either vancomycin or fidaxomicin can be used to treat mild-to-moderate CDI using criteria established by the 2017 IDSA/SHEA Guidelines.[199] Metronidazole is inexpensive and may be used for first-episode mild-to-moderate CDI if vancomycin or fidaxomicin is contraindicated or not accessible, but cure rates are inferior and prolonged treatment beyond 10 to 14 days may be required. Vancomycin is also inexpensive if the intravenous form of the drug is formulated for oral administration in flavored syrups to disguise the unpleasant taste, as is done in most hospital pharmacies. Vancomycin capsules for oral administration are available from multiple generic suppliers in the United States; however, their cost remains high compared to the liquid preparations.[206,210] Other agents have been used for CDI treatment, including nitazoxanide, rifaximin, teicoplanin, and bacitracin, but none is FDA approved for CDI nor has demonstrated any advantage over vancomycin or fidaxomicin, and some are not available for use in the US market.

Severe *Clostridioides difficile* Infection

Although there is no general agreement about which patients have indications of severe disease at treatment onset, the most predictive biomarkers are WBC >15,000/μL and increase in serum creatinine >1.5 times baseline or an absolute creatinine >1.5 mg/dL if prior creatinine level is not available.[140,199] Additional biomarkers include low serum albumin (<2.5 mg/L) and elevated C-reactive protein.[99,141] Severe outcomes are also more common with infection caused by the NAP1/BI/027 and NAP7-8/BK/078 strains of *C. difficile,* but it is not recommended that treatment decisions be based on strain type, since patients exhibit a full range of severity with these strains, from asymptomatic colonization to fulminant CDI, and treatment decisions should be based on the biomarkers of the individual patient, not the strain type of the infecting organism. Vancomycin is the preferred treatment agent for patients with severe CDI. Fidaxomicin may be a suitable alternative to vancomycin because it demonstrated comparable treatment response to vancomycin and lower rates of recurrence; however, the cost of fidaxomicin is considerably higher than vancomycin, particularly if the inexpensive liquid vancomycin formulation is used.[210] Both vancomycin and fidaxomicin have lower cure rates and higher recurrence rates with patients infected by the NAP1/BI/027 strain of *C. difficile* compared to other *C. difficile* strains, so there appears to be no advantage to either drug for treating CDI caused by these strains.[142]

Severe Complicated or Fulminant *Clostridioides difficile* Infection

Severe complicated or fulminant CDI is defined as a CDI patient with hypotension or shock, ileus, or toxic megacolon. These patients are the most difficult to treat and have the highest risk of mortality or need for surgical intervention to manage CDI. Recommendations for treatment of these patients in the 2017 IDSA/SHEA Guidelines are supported by only low-to-moderate evidence (see Table 243.4). If possible, these patients should be cared for in an intensive care unit for fluid and

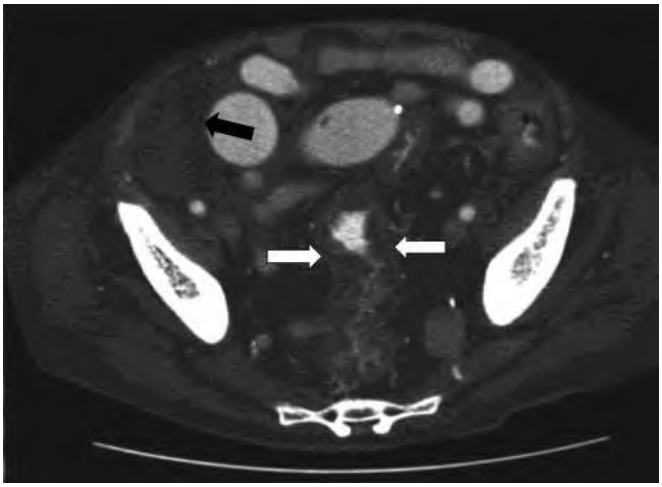

FIG. 243.7 Computed tomography scan of patient with pseudomembranous colitis demonstrating ascites *(black arrow)* and mucosal thickening of the colon *(white arrows).*

blood pressure support and have surgical consultation early in the course of illness to prepare the surgical team for the possible need for urgent colectomy or ileostomy in the event medical management is not successful. These patients often have an acute abdomen, and computed tomography scanning reveals typical findings of a diffusely thickened colonic wall and ascites (Fig. 243.7). Because of the presence of ileus in many of these patients, there is concern that vancomycin given orally or through a nasogastric tube may not reach the colon. As a result, the recommended dose of vancomycin is increased to 500 mg 4 times a day and addition of intravenous metronidazole is also recommended. Additional vancomycin can also be given via rectal retention enema (500 mg in 100 mL normal saline every 6 hours), if complete ileus is present. Anecdotal data indicate that tigecycline intravenously may be an effective alternative to intravenous metronidazole. The tigecycline minimal inhibitory concentrations range from 0.016 to 0.25 mg/L for *C. difficile,* and through primary biliary excretion of unchanged drug, up to 59% of the intravenous dose is recovered in feces. The number of successfully treated patients is very small, there has already been a failure reported, and tigecycline is not FDA approved for treatment of CDI.[211,212] Other strategies for fulminant CDI not responding to treatment include intravenous immunoglobulin, for which there has never been a randomized trial and for which evidence of benefit is poor.[213]

Usual surgical indications for CDI have included perforation, toxic megacolon, and acute abdomen, but failed medical management of patients with hypotension or shock is also an indication for colectomy. Patients should have WBC and lactate levels monitored at least daily as an indicator of possible need for surgical intervention. Surgical outcome with subtotal colectomy is more successful if patients undergo resection before the serum lactate rises to 5 mmol/L or the WBC count to 50,000/μL.[214] The mortality following subtotal colectomy for CDI often exceeds an unacceptably high 50%, especially if surgery is performed in patients with lactate levels >5 mmol/L or WBC counts >50,000/μL.[214,215] As a substitute for colectomy, a diverting ileostomy procedure followed by colonic lavage with warmed polyethylene glycol solution and daily vancomycin via the ileostomy has been evaluated in 42 patients. This operation was accomplished laparoscopically in 35 patients (83%). Ileostomy resulted in reduced mortality compared to a historical control population who had undergone colectomy (19% vs. 50%; odds ratio, 0.24; $P = .006$). Preservation of the colon was achieved in 39 of 42 patients (93%).[215] These early results are encouraging because of not only the possibility to reduce mortality, but also colon preservation.

First Recurrence of *Clostridioides difficile* Infection

First recurrences of CDI have usually been treated with the same antimicrobial regimen as used for the primary CDI episode, and responses to metronidazole and vancomycin are comparable, with 33.3% risk of a subsequent recurrence.[216] Recurrence is not thought to reflect an antimicrobial failure, but rather is a failure of the host to either reconstitute his or her microbiota to establish colonization prevention, or failure of the host to develop a protective antitoxin antibody immune response during the initial infection, or both. The 2017 IDSA/SHEA Guidelines recommend treatment of first recurrence of CDI with either vancomycin for 10 days, vancomycin for 10 days followed by taper and pulse dosing, or fidaxomicin for 10 days[199] (see Table 243.4). All are weak recommendations with low-to-moderate quality evidence, reflecting the lack of definitive data for management of recurrent CDI. Fidaxomicin has been shown to be comparable to vancomycin for treatment of first CDI recurrences, with a response rate of >90% for each following a 10-day treatment course, but fidaxomicin was superior to vancomycin with a significantly reduced rate of subsequent recurrence within 28 days (vancomycin, 35.5% recurrence; fidaxomicin, 19.7% recurrence; $P = .045$).[217] This study requires confirmation in a larger population given the marginally significant probability, but avoidance of a subsequent recurrence occurred in over 80% of fidaxomicin-treated patients, which is encouraging and could justify the higher acquisition cost of fidaxomicin. More recently, the standard dose of fidaxomicin 200 mg twice a day for 10 days has been changed to 200 mg twice a day for 5 days followed by 200 mg every other day for an additional 20 days for treatment of both first episode and first recurrence of CDI, and although overall sustained cure (treatment success without recurrence) was not improved over standard dosing, the recurrence rate was reduced to less than 5%.[218]

Multiple Recurrences of *Clostridioides difficile* Infection and Fecal Microbiota Transplantation

For patients who have had a second recurrence of CDI, there is no proven effective treatment regimen. Guidelines advise avoiding metronidazole because of the potential for development of neuropathy following prolonged use, and recommend a vancomycin-tapering regimen, one example of which is listed in Table 243.4. Although there is no standardized regimen for duration or rapidity of tapering or pulse dosing, the addition of every-third-day pulse dosing has been shown in an observational study to have a higher success rate than every-other-day pulse dosing (81% vs. 61%; $P = .03$).[219] Most strategies for prevention of recurrence in patients who have had multiple recurrences utilize a longer than usual treatment course with gradual reduction of dose, or additional treatment following 10 days of vancomycin with an antimicrobial that will have a presumably reduced impact on the normal microbiota, such as rifaximin or fidaxomicin.[220,221] Although results are encouraging with these so-called chaser regimens (vancomycin followed by rifaximin or fidaxomicin), there are no controlled, randomized, or blinded studies on which to compare efficacy.

Interest in the use of microbiota replacement therapy, typically referred to as fecal microbiota transplantation (FMT), has increased dramatically in the past few years. The initial randomized comparative study of FMT was done in comparison to treatment with vancomycin at a high dose (500 mg 4 times/day for 14 days, and the same vancomycin dose plus bowel lavage).[222] The study was discontinued early due to the superiority of the FMT regimen, which was given via nasoduodenal tube. Patients in the study had experienced a median of two to three (range, one to nine) previous recurrences of CDI. The first FMT resulted in resolution of further CDI episodes in 81% of patients. In comparison, patients treated with vancomycin had a 31% response ($P < .008$), and those treated with vancomycin with bowel lavage had a 23% response ($P < .003$).[222]

A systematic review in 2015 summarized 2 randomized controlled trials, 28 case series, and 5 case reports that described FMT for treatment of *C. difficile* infection.[223] For the 516 patients who were treated for recurrent CDI, FMT was highly effective, showing disease resolution in 85% of cases. In 2017, another group focused on the 10 randomized controlled trials that examined FMT for recurrent CDI, 5 of which compared FMT to either placebo or vancomycin treatment and the other 5 of which compared FMT delivery modalities.[224] Again, FMT was shown to be more effective than placebo or vancomycin, although

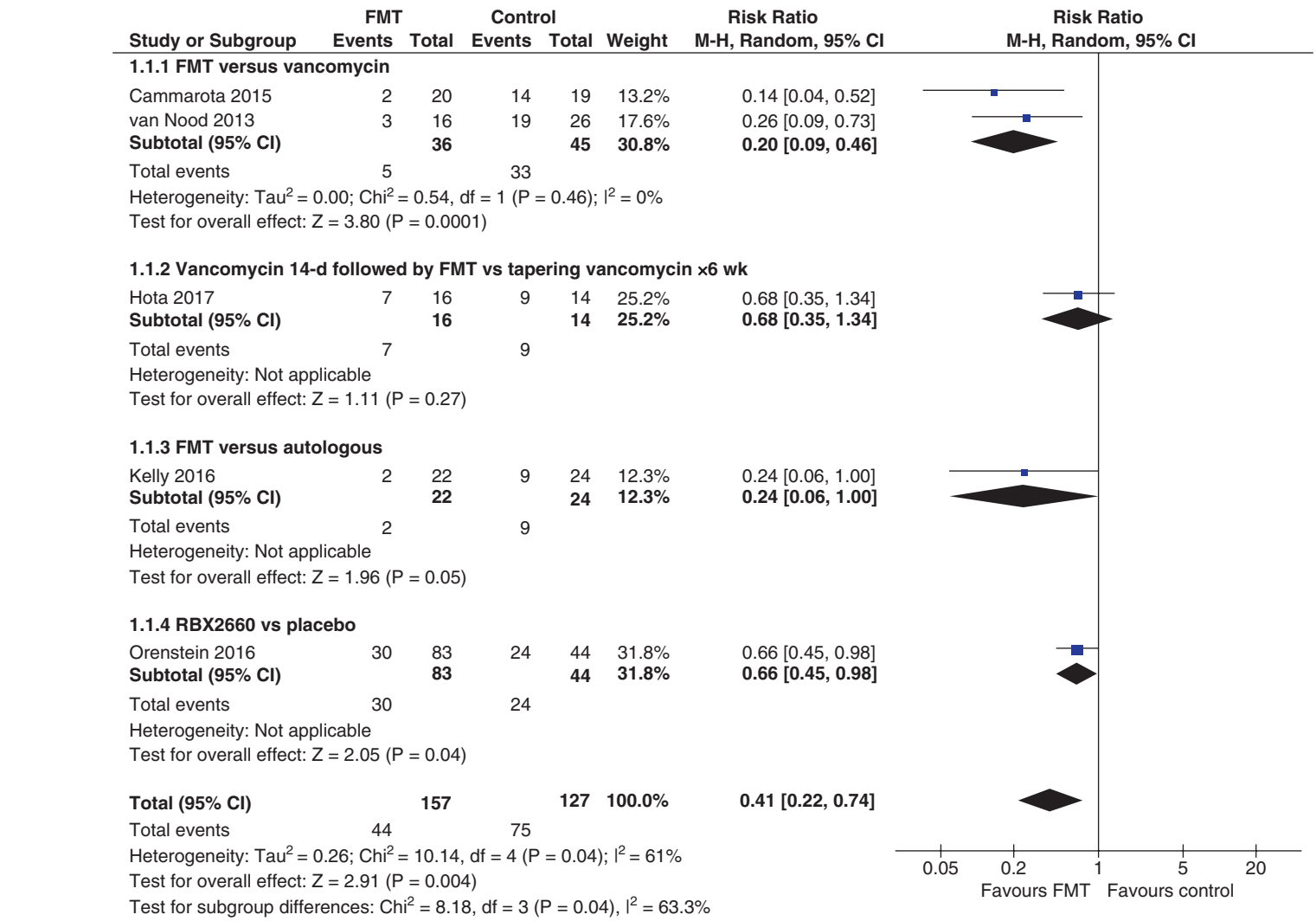

FIG. 243.8 Results of fecal microbiota transplantation *(FMT)* for recurrent *Clostridioides difficile* infection. A recent systematic review of controlled trials of FMT for recurrent *C. difficile* infection concluded that there was moderate-quality evidence supporting the use of FMT over vancomycin or placebo. *(From Moayyedi P, Yuan Y, Baharith H, Ford AC. Faecal microbiota transplantation for* Clostridium difficile*-associated diarrhoea: a systematic review of randomised controlled trials.* Med J Aust. *2017;207:166–172.)*

there was considerable variation between studies (Fig. 243.8). This study as well as another recent meta-analysis showed that both fresh and frozen fecal material had equal potency when used for FMT.[225] Lyophilized fecal preparations also appear to be another effective alternative for the preparation of material for FMT.[226] It should be noted that significant questions remain regarding methodology for FMT. In part this is due to the fact that many reports fail to describe key methodologic components in detail.[227]

The results of a European consensus conference on the clinical use of FMT was published in early 2017.[228] Although this group considered the use of FMT for other clinical indications, they concluded that they could only recommend FMT for CDI. FMT was recommended not only for multiple recurrences of CDI (defined as return of symptoms within 8 weeks after the onset of a previous episode that resolved after completion of initial treatment) but also for CDI not responding to standard therapy (vancomycin) for at least a week, and severe *C. difficile* colitis with no response to standard therapy after 48 hours.[229]

The clinical success of FMT reinforces the treatment benefit of reestablishing the indigenous microbiota in patients, thus restoring colonization resistance. Following FMT, it has been demonstrated that the gut microbiome of the recipient rapidly changes and resembles that of the donor.[230,231] Because recurrent CDI is associated with decreased overall diversity of the gut microbiome,[89] it is instructive to note that successful treatment with FMT is accompanied by an increase in microbiome diversity.[230,232]

The FDA has determined that the use of FMT requires filing an investigational new drug application, although there is a statement that there will be enforcement discretion in the setting of FMT for the clinical indication of treating recurrent CDI. So-called stool banks are providing stool preparations from screened donors for physician purchase and use for FMT CDI treatment under enforcement discretion. There have also been efforts to prepare fecal material for FMT utilizing clinical good-manufacturing processes to produce a stable, standardized, and reproducible product for clinical use. One of these formulations (RBX2660) was used in a prospective, multicenter open-label study and shown to be effective in treating recurrent CDI.[233] In an alternate approach, another commercial entity has prepared feces by treatment with ethanol to eliminate potential pathogens and to select for spore-forming organisms. This product (SER-109) was used to successfully treat patients with recurrent CDI in an open-label trial but failed to show superiority over placebo in a randomized blinded trial.[234]

As an alternative to FMT, there is also work on the development of treatment in the form of specific cultivars of the indigenous gut microbiota for the treatment of recurrent CDI. Initial work in continuous-flow cultures and germ-free mice demonstrated that cultivars from the hamster cecum could successfully compete with *C. difficile*.[235] More recent work

in murine models of CDI have shown that individual members and defined combinations of the indigenous mouse gut microbiome can restore colonization resistance against CDI.[236,237] The value of this approach was demonstrated in two patients who were given a mixture of 33 bacterial cultivars isolated from a healthy donor for the successful treatment of recurrent CDI.[238]

Monitoring Outcome of Treatment

It is important to monitor response to treatment at a minimum daily basis for mild, moderate, and severe CDI patients, and multiple times per day for fulminant CDI patients. A clinical response is usually evident within the first 3 days of treatment and includes improvement in abdominal pain, reduced frequency of stools, decreasing WBC count if elevated, and resolution of fever if present. If patient symptoms are worsening or have not improved by day 5 or 6 of treatment, a change of therapy is indicated. Usually the therapy change is from metronidazole to vancomycin, but there is no advantage to using both agents together orally. Stool frequency should decrease to ≤3 per day for at least 2 days by end of therapy. Successful treatment is measured by resolution of clinical symptoms, and not by microbiologic retesting of stools, which in the majority of successfully treated patients will still harbor *C. difficile* for 6 or more weeks following treatment. The administration of additional antibiotics for non–*C. difficile* infections should be avoided if at all possible during and after CDI treatment because they lead to a decreased response to treatment and an increased risk of recurrence.[136]

After successful treatment, patients should be informed of the possibility of recurrence of symptoms, usually within the first month following treatment, and to call their health care provider if diarrhea symptoms (>3 loose or unformed stools per day) recur, since recurrent CDI can be severe or fulminant and require rapid diagnosis and treatment. Stool testing should be repeated to document toxin in stools if possible, because retention of the organism (and NAAT and GDH test results) will commonly be positive following CDI treatment. Re-treatment should follow the recommendations in Table 243.4. Finally, irritable bowel symptoms may occur following CDI and can be difficult to distinguish from recurrent episodes.[239]

CONCLUSIONS

The epidemiology of CDI continues to evolve as it is now apparent that transmission of *C. difficile* strains occurs internationally and that strains of *C. difficile* possessing binary toxin in addition to toxin A and B are the cause of higher patient mortality. The appearance of new *C. difficile* strains is a dynamic process, with introductions to health care facilities of these new strains occurring frequently, reflecting in part the unremitting selective pressure of extensive antibiotic use. It should be anticipated that this will result in new health care–associated outbreaks caused by previously unrecognized strains and emphasizes the need to maintain a high level of vigilance through molecular typing of strains to detect these events.

Diagnosis, prevention, and treatment of CDI are presently in a state of flux, with promising new modalities on the horizon. Availability of highly sensitive NAAT and GDH algorithms should improve infection-control efforts by identifying the correct patients for isolation; however, further study to determine the best diagnostic testing algorithm is needed so that we do not unnecessarily treat patients who may be colonized with *C. difficile* but have diarrhea for another reason.

Improved treatment and prevention is being approached through biotherapeutics, immunologics, and vaccines (see Fig. 243.5). These include use of live bacterial organisms to restore colonization resistance, such as nontoxigenic *C. difficile* to prevent primary and recurrent CDI and use of FMT as an effective treatment of CDI to restore the microbiota. It is anticipated that at some point a subset of bacterial organisms in the microbiota will be identified that will provide the same benefit as FMT and will be available for oral administration. As early as 1989 a combination of bacterial organisms was identified that was effective in treating recurrent CDI.[240]

We now have available an intravenous monoclonal antibody, bezlotoxumab, directed at toxin B of *C. difficile* that in two large phase III clinical trials in 2655 adults with first-episode or recurrent CDI was shown to reduce recurrence of CDI from 27% to 17% ($P < .001$).[76] Actoxumab, a monoclonal antibody directed against toxin A, was ineffective alone and was not significantly better than bezlotoxumab alone when used in combination with bezlotoxumab. Patients with predefined high risks for recurrent CDI (age ≥65 years, severe CDI, immunosuppression, history of CDI) were most likely to benefit from bezlotoxumab, but those lacking any risk factors did not benefit.[241] Subjects with multiple risk factors benefitted most. Finally, multiple vaccines are being developed for prevention of CDI utilizing antigens derived from *C. difficile* toxins. These vaccines appear to be safe and are highly effective in animal models, but a phase III CDI vaccine clinical trial has recently been discontinued for futility following an interim analysis, suggesting that a successful vaccine for CDI may be more difficult to achieve than anticipated.

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The complete reference list is available online at Expert Consult.

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