CLOSTRIDIUM DIFFICILE 027:  
The Recent Emergence of a New Strain

Course # DL-990

by

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Approved for 3.0 CE
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Level of Difficulty: Intermediate

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CLOSTRIDIUM DIFFICILE 027:
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OUTLINE
A. Introduction
B. History of Clostridium difficile Infection
C. Transmission of C. difficile
D. Illness/Symptoms
E. Microbiology of C. difficile Infection
F. Diagnosis and Identification of C. difficile Infection
G. Treatment
H. Prevention
I. Case Study
J. Conclusions
K. References

COURSE OBJECTIVES
After completing this course, the participant will be able to:
1. outline the history of C. difficile infection.
2. discuss the reemergence of C. difficile infection in the U.S.
3. explain the pathogenicity of the new toxigenic strain.
4. outline the clinical features of C. difficile infection.
5. explain how the organism or toxin is identified.
6. state methods to prevent C. difficile infection.

A. INTRODUCTION

Clostridium difficile is an anaerobic, gram-positive, spore-forming bacillus. The species name “difficile” was given to the organism because it was initially difficult to isolate. Subsequently, improved media and improved anaerobic techniques have allowed C. difficile to be more easily isolated. Some strains of C. difficile can be non-toxigenic, non-virulent, and can be part of the normal intestinal flora in about 3% of the population. These patients are asymptomatic, but are carriers of the organism (1).
The toxigenic strains, however, produce a spectrum of gastrointestinal disease ranging from mild diarrhea through moderately severe disease characterized by watery diarrhea, abdominal pain and cramps, nausea, fever, hypotension, sepsis, and even fatal pseudomembranous colitis. *Clostridium difficile* is recognized as the primary cause of nosocomial (hospital-acquired infection or HAI) infectious diarrhea in the majority of healthcare facilities (1).

Many earlier studies made reference to the disease or infection associated with *C. difficile* as *C. difficile* associated disease, or CDAD. However, in most current studies the infection is now described as *C. difficile* associated infection, or CDAI. The reader should be aware that both terms may be used interchangeably, although CDAI is more accurate.

The precipitating event for *C. difficile* to cause disease is disruption or alteration of the normal colonic microflora. This disruption usually is caused by broad-spectrum antibiotics, with clindamycin, cephalosporins, and broad-spectrum penicillins, including ampicillin and amoxicillin, most commonly implicated. Antibiotics with a reduced propensity to induce infection include aminoglycosides, metronidazole, and vancomycin. The risk of patients’ developing antibiotic-associated diarrhea with *C. difficile* increases substantially with longer than three days of antibiotic therapy in a medical care facility.

After disruption of the colonic microflora, colonization of *C. difficile* generally occurs when a patient ingests spores obtained from the healthcare environment. The loss of normal bacterial flora in the bowel, due to the action of the antibiotic, allows *C. difficile* spores to germinate into vegetative cells and produce toxins in the colon. Pathogenesis primarily involves the action of two toxins: an enterotoxin (toxin A), and a cytotoxin (toxin B) which are encoded by certain genes. Depending on host factors, either an asymptomatic carrier state or a clinical manifestation of *C. difficile* colitis can develop. CDAI can occur up to eight weeks after the discontinuation of antibiotics. Most cases, however, of in-patient *C. difficile* infection occur on days 4 through 9 of antibiotic therapy (2).

Risk factors for acquiring the disease are: being hospitalized, being on antibiotics while hospitalized, age (>65 years), number and severity of underlying diseases, gastric acid reducing drugs (such as proton-pump inhibitors), and faulty immune response to *C. difficile* toxins. Patients at highest risk for fulminant disease include those who recently received immunosuppressive therapy, recently underwent surgical procedures, or have a previous history of *C. difficile*-associated diarrhea. See Table 1, Risk Factors for Clostridium difficile-Associated Infection.

For many years, CDAI was considered a nuisance, causing mild diarrhea in older patients in hospitals and nursing homes. It was easily treated by either stopping the antibiotic the patient was taking, if possible, or by treating with either oral metronidazole or vancomycin. However, since 2000, there has been an increase in *C. difficile* cases as well as an increase in the morbidity and mortality associated with the disease. With the increased frequency and severity of CDAI, *C. difficile* now rivals methicillin-resistant *Staphylococcus aureus* (MRSA) infection as the most common healthcare-acquired infection in many hospitals (5).
The dramatic increase in the incidence and severity of healthcare-associated C. difficile infections is because of the emergence of a new strain of C. difficile that has been implicated as the cause of numerous epidemics in the USA, Canada, Europe, and Asia. This new strain, or ribotype, is called Clostridium difficile NAP1/BI/027, or simply C. difficile 027. With the advent of this new strain, and the increased incidence, severity, and mortality of C. difficile-associated disease, as well as the limitations of currently available therapeutic options, it is imperative that healthcare personnel take steps now to prevent transmission of C. difficile within healthcare facilities.

This Distance Learning Course presents the history of C. difficile infection, its mode of transmission, the clinical symptoms of the disease, the microbiology of C. difficile infection, the organism’s pathogenesis, methods for identifying the organism and its toxins, discussion of the new strain, treatment of C. difficile infections, and finally, methods for preventing CDAI.

B. HISTORY OF CLOSTRIDIUM DIFFICILE INFECTION

Initial research of C. difficile during 1974-1978 revealed that it was the primary cause of antibiotic-induced pseudomembranous colitis in health care facilities. The pathogenic role of C. difficile was established, risk factors were defined, and criteria for diagnosing and treating this disease were developed. A tissue cell cytotoxicity assay as the diagnostic test provided accurate results, and treatment with oral metronidazole or oral vancomycin was found to be highly effective. The disease at this time was associated with the patient’s taking an antibiotic, usually clindamycin. During the 1980s and 1990s, C. difficile infections were still usually benign and resulted in simple, easily treated diarrhea, generally in older patients in hospitals and nursing homes.

Additional diagnostic tests were introduced during the 1980s, such as enzyme immunoassays (EIAs) testing for toxin A or B, which became the standard for most laboratories because EIAs were less expensive, and faster and easier than cell cytotoxicity assay. Metronidazole became the favored treatment because it was less expensive and quelled fears of colonization by vancomycin-resistant organisms. Also in the 1980s, cephalosporins replaced clindamycin as the major inducers of C. difficile-associated infection because cephalosporins were so extensively used and had broad-spectrum capabilities. There was a reported relapse rate during this period of about 5-10% that was usually addressed by changing the patient to vancomycin therapy.

The picture of CDAI being a mild “nuisance,” however, has changed over the last 5-7 years. Now, CDAI has increased in frequency and severity throughout North America, Europe, and Asia and is a major nosocomial infection causing major epidemics. This recent dramatic increase is associated with the emergence of the hypervirulent toxin strain of C. difficile. The new strain can cause severe morbidity and mortality within a few days, often requires closing of nursing units, and leaves some patients in intensive care units facing surgical intervention. This
transformation challenged the entire approach to treating and preventing this serious infection. It is believed that overuse of antibiotics allowed the bacteria to develop resistance, creating the new toxic type.

In the 1980s to late 1990s, 84% of *C. difficile* isolates from patients were classified by PCR techniques as ribotype 001. However, since 2002, 80% of outbreaks have been spread by the newly recognized *C. difficile* strain identified as NAP1/BI/027. This strain is characterized by specific molecular techniques as NAP1 (North American pulsed-field type 1 strain based on its pulsed-field gel electrophoresis pattern), type BI (by restriction endonuclease analysis), and ribotype 027 (by PCR ribotyping). This strain can be further characterized as toxinotype III (by PCR characterization of the pathogenicity locus) (3).

The NAP1/BI/027 strain, often called ribotype 027, carries an extra toxin, known as binary toxin (or referred as CDT, *Clostridium difficile* toxin), in addition to toxins A and B. Binary toxin appears to be an additional factor that contributes to the strain’s virulence by inducing the formation of microtubule-based protrusions on the surface of the host epithelial cells that lead to an increased adherence of *C. difficile*.

Researchers have found that the new *C. difficile* 027 strain has several mutations in a critical toxin regulatory gene that normally reduces the production of toxins A and B. In the absence of a functional suppressor gene, the 027 organism produces hyper amounts of toxins A and B, leading to severe morbidity and mortality. Further, the new strain is more resistant to fluoroquinolones, including antibiotics such as ciprofloxacin, norfloxacin, and levofloxacin (3).

Historically, the reported incidence of CDAI in American hospitals from 1987 to 1998 was approximately 10 infections per 1,000 admissions. More recent investigations of hospital outbreaks in the United States report the incidence of *C. difficile* infection has increased from 11 infections per 1,000 admissions (1999 to 2002) to 18 infections per 1,000 admissions (2005-2014) (4,5). Data from the United States, Canada, Great Britain, and throughout Europe have shown that the overall infection rate of *C. difficile* more than doubled between 2000 and 2005 (4,5). In 2014, for example, more than 490,000 cases of CDAI were recorded in U.S. hospital discharge records, and 29,000 infected people died (4,5). Eighty percent of deaths were associated with Americans 65 years and older, and 100,000 of the 490,000 cases developed among residents of U.S. nursing homes (4,5).

In an outbreak in 2005 involving multiple hospitals in the province of Quebec, Canada, the incidence of *C. difficile* was 22.5 infections per 1000 hospital admissions, and the associated mortality was from 7% to as high as 16.7%. Fatality associated with this disease has also increased in the U.S. The number of death certificates indicating *C. difficile* infection as a cause of death increased nearly four-fold from 2000 to 2014, and some recent studies from 2014 show *C. difficile* fatality rates between 5-16% (4), compared with 1-2% from 1980-1999.

In November 2013, the Association for Professionals in Infection Control and Epidemiology (APIC) and the Centers for Disease Control and Prevention (CDC) reported that the incidence of *C. difficile* infection is far more common in U.S. hospitals than healthcare workers thought (5). The APIC and CDC reports stated that as many as 13 out of every 1,000
U.S. hospital patients are infected with *Clostridium difficile*, meaning that approximately 7,178 inpatients on any given day were infected or colonized with *C. difficile*. The CDC report estimated that on any given day these infections cost between $17.6 million to $51.5 million and killed between 165 and 438 patients. The APIC data show that approximately 336,600 patients contracted *C. difficile* in healthcare facilities in 2011. The highest rates of CDI-related hospital stays were in the Northeast, followed by the Midwest, South, and West regions (4,5).

The financial impact of CDI on individuals and healthcare institutions in America is considerable. One follow-up in 2010 of 271 patients at a teaching hospital in Boston determined that hospital-acquired CDI resulted in a median 54% increase in hospital costs (an additional $4,800 per hospital admission) and a median 3.6-day longer hospital admission compared to patients without CDI. The data also showed that there was an increase of $13,655 to $18,067 per case for recurrent CDI. Other data presented in 2007 showed that length of hospital and ICU stay were approximately twice as long for patients with *C. difficile*-associated infection, compared with patients without any CDI. As the U.S. population becomes older and frailer, more patients are at risk of serious *C. difficile* infection, further impacting the healthcare system. *C. difficile* associated infection places a significant economic burden on the U.S. health care system. The acute-care direct cost of CDI in the U.S. is estimated to be $4.8 billion in 2014. Average cost for a CDI stay was $24,400 in 2009, estimated to be 2.3 percent of all hospital costs in the U.S. Current hospital stay for CDI is 16.0 days and costs $31,500 per patient (6,7).

A recent epidemiologic twist of *C. difficile* infection is the appearance of the organism throughout the entire hospital population, not just primarily in the ICU as has been true in the past. Historically, *C. difficile* has been a healthcare system-associated infection among elderly (>65-year-old) and high risk populations, such as intensive care patients. Although this is still largely the case, patients previously categorized as low risk in hospitals, such as pediatrics and maternity patients, are now also being affected, and these are patients in whom the disease may be life-threatening (6).

Another recent trend is an increase of *C. difficile* infection from patients in the community (7). More than 150,000 of the half of million infections in 2014 were community associated and had no documented inpatient health care exposure, however, the study found that 82 percent of patients with community-associated *C. difficile* infection reported exposure to outpatient health care settings such as doctor’s or dentist’s office in the 12 weeks before their diagnosis and were given antibiotics (7). These patients from the community generally have no risk factors other than being on an antibiotic, with clindamycin being the antibiotic most commonly prescribed. Also, among these community group populations, person-to-person transmission has been reported, including cases in children (7).

The new, more virulent *C. difficile* 027 strain has been reported in pork production operations in the United States, Canada, and Europe, and can be commonly associated with disease in neonatal pigs (6). A similar strain can be commonly recovered among diseased dairy calves in the United States (6). Like the human epidemic strain, the *C. difficile* 027 strain found in animals has many pathogenic characteristics and produces binary toxin. This suggests that
domestic animals, by way of retail meats, may be one possible source of *C. difficile* infection in the community.

The true incidence of *C. difficile* infection, and the infection rate due to the hypervirulent *C. difficile* NAP1/BI/027 strain, however, is not known. In the majority of cases, the diagnosis of *C. difficile* infection is made by using EIA to test just for the toxin. Therefore, the organism itself is not characterized by molecular typing techniques to allow correlation of incidence of disease with a particular strain type. Also, since many hospitals perform only stool toxin testing (EIA), the true incidence may be actually higher because of the reported false negative rate associated with the performance of the test (approximately 40% of diagnoses are missed by insensitive methods). To further complicate the issue in the U.S., in the majority of states the diagnosis of *C. difficile* infection is not reported to public health authorities to correlate incidence. According to an email this author received from the CDC, “The decision to make a particular disease reportable is made by each state.” In California, for example, *Clostridium difficile*-associated disease was not previously a reportable disease. However, beginning January 1, 2009, hospitals are now required to report to the California Department of Public Health their rates of hospital-onset *Clostridium difficile*-associated infection. California’s Department of Public Health (CDPH). Data from this report first became available in 2010 shows the incidence of *C. difficile* infection in hospitals and clinics. See Table 2. Hospital-Onset *Clostridium difficile* Infections Reported by California Hospitals by Year (2010-2014) (14). See this report to find out how your hospital, or a competing hospital, is doing in the number of cases of *C. difficile* per year. Although the CDPH states in their first public health surveillance report on *C. difficile* infection that the data is not perfect and more work needs to be done concerning the quality and its completeness of the report, it is a step in the right direction to assess how hospitals are managing to control the spread of infection.

In January 2013, the Centers for Medicare and Medicaid Services (CMS) began requiring acute care hospitals participating in their Hospital Inpatient Quality Reporting Program to begin reporting *C. difficile* for all patients. In 2015, CMS quality reporting programs required *C. difficile* reporting from long-term acute care hospitals and inpatient rehabilitation facilities.

### C. TRANSMISSION OF *C. DIFFICILE*

*C. difficile* is easily passed from patient to patient. It is transmitted by spores from feces of infected patients, primarily in a healthcare environment. *C. difficile* spores have been recovered from floors, windowsills, toilets and faucets, as well as the hands of hospital workers caring for infected patients. The spores of *C. difficile* are difficult to destroy using customary antibacterial agents, including alcohol-based hand disinfectants, and are able to survive for months on most environmental surfaces. The spores are resistant to heat, radiation, chemicals, and antibiotics, making a contaminated environment difficult to clean. One recent study found that spores were able to survive up to 40 days on environmental surfaces after infected patients had been discharged (7). Any surface, device, or material that becomes contaminated with feces may serve as a reservoir for the *C. difficile* spores, including commodes, bathing tubs, electronic
rectal thermometers, stethoscopes, blood pressure cuffs, and other equipment. Several studies report that spores have been recovered from bed rails, bed tables, privacy curtains, and call buttons (7). In another study, *C. difficile* was found on the hands of nearly 60 percent of doctors and nurses caring for infected patients, and 20% of nurses’ uniforms had *C. difficile* spores by the end of a workday (6). When patients touch contaminated surfaces and then pick up food or put their hands in their mouth without washing their hands first, they ingest the spores and are in danger of developing the disease.

Routine cleaning is not sufficient to destroy the spores of *C. difficile*. Researchers at the Cleveland VA Medical Center found that after routine cleaning at a hospital, 78% of many environmental surfaces were still contaminated (7). To kill the spores of the *C. difficile* organism, meticulous cleaning with bleach is imperative. Hand washing presents a problem because alcohol-based sanitizers do not kill the spores. Hand washing with soap and water seems to remove the bacterial spores more efficiently than alcohol-based sanitizers, although the increased use of alcohol-based sanitizers has not been shown to increase the number of patients with disease. Judicious infection control practices, barrier precautions, and private washrooms for infected patients are essential to prevent other patients from ingesting *C. difficile* spores and developing symptoms.

Data from the Association for Professionals in Infection Control and Epidemiology (APIC) in 2011 show that most hospitalized patients are exposed to the spores of *C. difficile* within 3 days of hospitalization. The rate of *C. difficile* acquisition is estimated to be 13% in patients with hospital stays of up to two weeks and 50% in those with a hospital stay longer than four weeks. Patients who share a room with a *C. difficile*-positive patient acquire the organism after an estimated hospital stay of 3.2 days, compared with a hospital stay of 18.9 days for other patients (4,5).

**D. ILLNESS/SYMPTOMS**

*C. difficile* is responsible for a spectrum of diseases, including asymptomatic carriage, uncomplicated diarrhea, moderate to severe diarrhea, pseudomembranous colitis (a severe infection in the colon in which the lining of the colon becomes inflamed and covered with exudates or plaques), and toxic megacolon (a life-threatening complication characterized by a very dilated and distended colon) that can, in some instances, lead to sepsis and death. See Table 3, Common Symptoms and Diseases Associated with *C. difficile* Infection. There can be histological changes in the colonic mucosa of the patient ranging from minimal inflammation to pseudomembranous colitis with plaques. Symptoms of *C. difficile* infection include watery diarrhea (at least three bowel movements per day for two or more days), fever, anorexia, nausea, and abdominal pain/tenderness. Patients often have an increase in their WBC count.

The three main risk factors for hospital-acquired CDAI are 1) being in a hospital, or having a lengthy hospital stay, 2) receiving an antibiotic (more than 90% of infections are associated with recent antibiotic use), and 3) being over 65 years old. Other risk factors for hospital-acquired CDAI include the presence of severe underlying disease, nasogastric
intubation, administration of enteral tube feedings, receipt of certain medications that change the pH of the GI tract or alter the normal bacterial flora, and gastrointestinal procedures or surgery. Also, among hospitalized patients, those being cared for on intensive care units (ICUs) are generally at higher risk compared with those being cared for on surgical services or other units. Patients in long-term care facilities are also at increased risk for CDAI. See Table 1, Risk Factors for Clostridium difficile-Associated Disease.

**Recurrent disease.** Recurrence is defined by complete abatement of CDAI symptoms while on appropriate therapy, followed by subsequent reappearance of diarrhea and other symptoms after treatment has been stopped. Recurrent CDAI often results from reinfection with the same or a different strain of C. difficile. Current studies using molecular methods have shown that up to one-half of recurrent episodes are reinfection rather than relapses of infection with the original strain. Recurrence may occur in approximately 25 percent of cases treated with metronidazole or vancomycin. Most recurrences present within one to three weeks after discontinuing antibiotic therapy, although recurrences rarely can occur as late as two to three months. Patients with at least one episode of recurrent C. difficile have a 45 to 65 percent chance of additional episodes (11). In patients 65 or older, a recurrence of C. difficile infection occurred 25-45 percent of the time, and these patients often died within 30 days of diagnosis of the recurrent infection (9).

**E. MICROBIOLOGY OF C. DIFFICILE INFECTION**

The intestines normally contain many good bacteria that help keep humans healthy by preventing invading microbial diseases from establishing in the colon. When a person takes an antibiotic, the antibiotic may kill or alter the ecology of the good normal flora in the colon. This may allow the spores of C. difficile to develop in the colon, grow into vegetative cells, and release toxins and produce disease. Experts also think that, in some cases, antibiotics themselves may actually cause these toxins to be released from the cells of C. difficile (6). When toxins are released, the colon becomes inflamed. Symptoms usually begin 4 to 10 days after a person begins taking antibiotics, but they might not develop until a few weeks after the person stops taking antibiotics.

Pathogenesis of C. difficile infection primarily involves the action of two clostridial toxins, toxin A and toxin B. Toxin A is an extremely potent enterotoxin that also has cytotoxic activity. (An enterotoxin alters the permeability of intestinal epithelial cells; a cytotoxin kills cells.) When toxin A damages the intestinal epithelial cells, the body produces an inflammatory response. Leakage from the damaged (or killed) epithelial cells draws macrophages and mast cells to the area, which in turn causes the production of inflammatory mediators (molecules released by immune cells to combat infection), cytokines and interleukins (signaling molecules), and leukocyte chemotaxis (white cells being drawn to the area). The body is unable to neutralize the toxin or prevent its action, so cellular debris builds up, fluids accumulate, cell damage continues, the body sends even more cells and molecules to the area, and the inflammatory response becomes a vicious cycle. The accumulation of the fluids and cellular debris results in diarrhea, and the profound colonic inflammatory response (colitis) is usually evidenced clinically
by a high WBC count. As colitis worsens, colonic ulcerations occur, and left unchecked, the accumulation of purulent and necrotic debris forms the typical pseudomembrane in the colon.

Toxin B has little enterotoxin activity, but is a potent cytotoxin. Therefore, toxin B produces the same inflammatory response described above, with the same outcomes. There is some evidence that compared to toxin A, toxin B is the major virulence factor. Some strains of \textit{C. difficile}, such as the new 027 strain, produce both toxin A and toxin B. Other \textit{C. difficile} strains may produce one or both of the two toxins, but these strains are still clinically relevant because they are capable of initiating the inflammatory response that leads to diarrhea and colitis.

Researchers have found that the salient features of the hypervirulent \textit{C. difficile} 027 strain are:

1. its ability to produce higher amounts of toxin A and toxin B than other clostridial strains. Further, \textit{C. difficile} 027 may also produce another toxin, called binary toxin, or CDT, which acts by inducing the formation of microtubule protrusions on the surface of host epithelial cells that permit increased adherence of \textit{C. difficile} (9).

2. an increased sporulation rate compared to other strains of \textit{C. difficile}. The increased sporulation rate of \textit{C. difficile} 027 allows the organism to survive and spread more readily in a hospital setting, leading to an increased incidence of hospital-acquired \textit{C. difficile} infection (8).

3. its ability to adhere more readily to human intestinal epithelial cells, compared with other \textit{C. difficile} strains (6). Normal bowel flora apparently prevent bacterial adherence. When normal bowel flora are disrupted by antibiotic therapy, efficient bacterial adherence may be one factor contributing to the establishment and predominance of the virulent \textit{C. difficile} 027 strain in hospitals where outbreaks have occurred.

4. its resistance to fluoroquinolones. This is of epidemiologic importance because fluoroquinolone antibiotics are used extensively in both inpatient and ambulatory settings to treat a wide variety of infections. With their broad-spectrum activity, fluoroquinolones are effective in altering the normal colonic flora and paving the way for either colonization with \textit{C. difficile} spores or providing an environment for the germination of existing spores within a patient’s colon. With extensive use of fluoroquinolones, this selective pressure will continue to increase the incidence of \textit{C. difficile} infection. An interesting finding is that in Europe taking the antibiotic piperacillin/tazobactam is a risk factor for acquiring \textit{C. difficile} infection.

Host immune response is important in determining the outcome of \textit{C. difficile} infection. It has been shown that the presence of low levels of serum IgG in response to toxin A are associated with recurrent disease. Asymptomatic carriers mount a protective immune response by producing a high level of IgG in response to toxin A. Patients with active CDAI, on the other hand, have a very low IgG level in response to toxin A, and thus no immunity. See Figure 1, Pathogenesis of \textit{C. difficile} Infection.

\textbf{F. DIAGNOSIS AND IDENTIFICATION OF \textit{C. DIFFICILE} INFECTION}
The clinical diagnosis of *C. difficile*-associated disease requires a patient history and laboratory tests to detect *C. difficile* and its toxins. The patient history should place particular emphasis on antibiotic use during the preceding two to three months. Other important factors to consider are a history of fever, immunosuppression, a recent surgical procedure, previous infection with *C. difficile*, recent changes in bowel habits (including diarrhea), a high WBC count, and the presence of other bowel irregularities. See Table 3, Common Symptoms and Diseases Associated with *C. difficile* Infection.

Generally, it is recommended that a patient who acquires diarrhea after three days of hospital admission should be tested only for the presence of *C. difficile*. Further, it is recommended that laboratory testing for *C. difficile* be performed on loose or watery specimens only. Generally, laboratory testing should not be performed on formed stool specimens due to the normal flora carriage rate of *C. difficile* in some patients.

The laboratory diagnosis of CDAI can be accomplished by a variety of methods. See Table 4, Methods and Tests for the Detection of *Clostridium difficile*, and Table 5, Performance of Various Testing Methods for *Clostridium difficile*. There are advantages and disadvantages to each of these categories of laboratory tests. These tests include:

1. tissue culture cytotoxicity assay (CTA)
2. bacterial culture to isolate the organism
3. detection of toxins by enzyme-linked immunoassays (EIAs) or other methods
4. detection of cellular antigens (GDH) and also by EIA methods
5. molecular amplification techniques.

Since the original *Clostridium difficile* Distance Learning Course was written in 2009, various testing methods previously recommended have been found to be unreliable in detecting toxigenic *C. difficile* in fecal samples. The major reason is that present day studies find the “gold standard” for *C. difficile* toxin lacks sensitivity (the tests do not identify the true number of positives). Researchers find that when a less sensitive new test is compared against a less sensitive “gold standard” test, the new test looks good and reliable, but in fact it is not. As data from studies becomes available, various gold standard tests that were initially adopted have been abandoned over the last few years, which have changed current recommendations for laboratory testing for *C. difficile*.

One test, the tissue culture cytotoxicity assay (CTA), was thought of as the gold standard for a number of years, but studies now show that CTA is only about 70% sensitive. See Table 5, Performance of Various Testing Methods for *Clostridium difficile*. Further, the cytotoxicity test is difficult to perform, and results are not available for 48-72 hours. This method requires the use of tissue cells (e.g., fibroblast or human foreskin), and detects the cytotoxic effect produced by the presence of toxin B on the cells. Antitoxin neutralization is usually performed to confirm the test results. However, due to lack of sensitivity, increased turnaround times, increased cost, labor, and facility requirements, and the effort of maintaining a tissue culture line, most clinical laboratories no longer use this method and it is not recommended as a “gold standard” test.
Another laboratory diagnostic method that was thought of as reliable is bacterial culture to isolate the organism. *Clostridium difficile* can be isolated from stool using special media. Stool culture for *C. difficile* is often associated with false-positive results because the culture is not specific for toxin-producing *C. difficile* strains and is only about 70% sensitive and about 90% specific. See Table 5, Performance of Various Testing Methods for *Clostridium difficile*. Further, stool culture for *C. difficile* is labor-intensive, requires the appropriate anaerobic environment to grow the organism, does not produce results for 48-72 hours, and does not differentiate between toxigenic and non-toxigenic strains. Therefore, stool culture for *C. difficile* is not generally clinically useful and has limited applicability except when epidemiological strain typing is needed, or when used in conjunction with another method that detects toxin production. If a stool culture is used, the medium most commonly used to isolate *C. difficile* is CCFA (cycloserine-cefoxitin-fructose agar). This medium can be purchased from various commercial vendors. To perform a culture, a stool specimen or fecal swab is inoculated directly onto CCFA and incubated anaerobically at 35 to 37°C for 18 to 24 hr. Colonies of *C. difficile* on CCFA are circular, yellowish to white with a ground-glass appearance. The colonies have a very distinctive odor of horse manure. In addition, *C. difficile* colonies on CCFA fluoresce chartreuse under UV light.

CHROMagar medium *C. difficile* (bioMerieux) was developed in 2014. It is a fluorogenic culture medium that was designed to speed up (24 hours) the culture and identification of *C. difficile*. This medium permits the isolation of *C. difficile* to allow strain typing and antimicrobial susceptibility testing if needed. *C. difficile* is fluorescent under UV light (365 nm) on the CHROMagar medium. The use of this technique, however, still requires an anaerobic environment and a delay of at least 24 hours.

In 2008, the most common laboratory test for diagnosing *C. difficile*-associated infection was an enzyme immunoassay (EIA) that detects toxins. At this time, the College of American Pathology (CAP) reported that 80% of U.S. hospitals used some type of toxin testing EIA system because of their low cost, their ease of use, and their ability to produce test results on batches of samples. However, new data shows that these tests are less reliable than originally reported and the most common testing method for *C. difficile* now in 2015 has changed as I will mention later in this paper. Some EIA kits detect toxin A, while others can detect both toxin A and toxin B. These tests provide results within two to six hours and have a specificity of 83 to 90%. The sensitivity, however, ranges from 33 to 80%, which means that 20 to 60% false-negative results can occur. See Table 5, Performance of Various Testing Methods for *Clostridium difficile*. Their reportedly low sensitivity and potentially false-negative results have led several authors to raise serious questions about the advisability of stand-alone EIA testing for *C. difficile*. Since the clinical impression is that *C. difficile* is frequently under-diagnosed by toxin detection methods, many physicians try to improve diagnostic accuracy by ordering multiple *C. difficile* tests either on the same day or subsequent days. Mistrust of negative EIAs leads to excessive re-testing, inappropriate treatment, and isolation of an unacceptable number of patients. Investigators have shown that same-day or next-day repeat EIA testing is neither cost-effective nor an improvement...
in patient care, and is certainly an additional expense to the laboratory. Stand-alone EIA tests for the diagnosis of \textit{C. difficile} infection are no longer recommended (1,9). Many labs are transitioning from toxin enzyme immunoassays (EIA) to nucleic acid amplification tests because the sensitivity of EIA tests perform poorly (12).

Another assay method used in the laboratory for the detection of \textit{C. difficile} targets the enzyme glutamate dehydrogenase (GDH). GDH is a marker present in all \textit{C. difficile} strains regardless of toxin producing ability. GDH antigen kits are available, either latex or EIA based, and test for the glutamate dehydrogenase protein. Some kits have been developed in which one or two testing methods specific for \textit{C. difficile} (i.e., toxin A or B and/or glutamate dehydrogenase) are available in the same test. Test results are generally available in less than 1 hr. Testing for glutamate dehydrogenase alone has also been found to lack adequate sensitivity, about 85%. Recent data has shown that glutamate dehydrogenase can cross-react with \textit{Clostridium sordellii} (12).

The GDH assay is rapid but fails to distinguish toxigenic from non-toxigenic strains, so it must be combined with an additional test for toxigenicity. Therefore, a two-step testing algorithm was originally recommended in the first version of the \textit{Clostridium difficile} Distance Learning Course as a “gold standard” in which both EIA and GDH testing was followed by testing positives with cell culture cytotoxicity (CTA), but this method has also been found to lack sensitivity (55-85% sensitive). See Table 5, Performance of Various Testing Methods for \textit{Clostridium difficile}. The original studies of EIA and GDH followed by testing positives with cell culture cytotoxicity (CTA) rested on the premise that the cytotoxin assay (CTA) was a reliable “gold standard” test. However, this is another example of an insensitive test being compared with a relatively insensitive “gold standard” test, making the results incorrect. While the initially recommended two step algorithm offered an improvement over stand-alone EIA testing or stand-alone GDH testing, it is not as sensitive as molecular testing (9).

Current recommendations from CDC (9) suggest that molecular amplification methods for the detection of the toxin gene, not the toxin itself, is the most sensitive and specific diagnostic method for \textit{C. difficile} infection. See Table 5, Performance of Various Testing Methods for \textit{Clostridium difficile}. The CDC’s current recommendations are based on studies that compare molecular amplification methods with toxigenic culture (a research protocol in which the stool sample is treated to enrich \textit{C. difficile} spores and then the sample is inoculated onto CCFA agar and colonies verified to produce toxin). Researchers find that molecular methods are fast and extremely accurate with a high sensitivity and specificity. Therefore, molecular methods are now the new recommended “gold standard” for the diagnosis of \textit{Clostridium difficile} infection.

There are currently various FDA approved molecular testing kits available. See Table 6, Molecular Tests for the Presence of Toxin-Producing \textit{Clostridium difficile}. In late 2008, the FDA approved a real-time PCR amplification kit for \textit{C. difficile}, named BDGeneOhm \textit{C. diff} Assay (BD Diagnostics, San Diego, CA), that targets the toxin B gene found in toxigenic \textit{C. difficile} strains. Real-time PCR provides amplification and simultaneous quantifying, providing much
faster results than normal PCR procedures. The assay uses PCR for the nucleic acid amplification of the toxin B gene and fluorogenic target-specific hybridization probes for the detection of the amplified DNA using a glass bead lysis system. Results with the BDGeneOhm system are available in about 1 hour 45 min. from sample to result. Three other real-time PCR kits were approved by the FDA in 2009. One was the Cepheid GeneXpert Assay/Epi that uses fully automated real-time polymerase chain reaction (PCR) with fluorogenic detection of the amplified DNA, providing results in 45 minutes. Another real-time PCR system approved by the FDA in 2009 was the GenProbe Prodesse ProGastro Cd Assay that uses an external extraction method and provides results in about 3 hours. In 2010, the FDA approved the Illumigene C. difficile Assay from Meridian Biosciences. This method uses the LAMP technology (loop-mediated isothermal amplification) that provides amplification at a constant temperature in a single step with samples and primers for testing in any size clinical laboratory. Results are available in 1 hour.

In 2012 the BD Max C. diff Assay was approved by the FDA, and in 2013 the Portrait Toxigenic CD Assay and the Quidel Molecular Direct C. difficile Assay were approved. While many previous molecular assays for Clostridium difficile were relatively complex and/or expensive, recent isothermal DNA amplification technologies such as the LAMP method used in the Illumigen C. difficile Assay, and the helicase-dependent amplification method (HDA) used in the Portrait Toxigenic C difficile Assay, are less complex and less expensive because they do not require a thermocycler or fluorescence reader. These new isothermal molecular tests offer laboratories that currently are using toxin immunoassays the benefit of the high level of accuracy of a molecular test without incurring the significantly higher costs associated with complex instruments and a highly trained molecular staff. See Table 6, Molecular Tests for the Presence of Toxin-Producing Clostridium difficile, and Table 5, Performance of Various Testing Methods for Clostridium difficile.

In 2013 the Verigene Clostridium difficile Nucleic Acid Test (Verigene CDF) (Nanosphere, Northbrook, IL) was approved by the FDA. This test utilizes a nanoparticle-based array hybridization method to detect Toxin A and Toxin B. In addition, the assay detects binary toxin gene associated with the epidemic strain ribotype 027. Studies show improved sensitivity and specificity when compared to either culture or to EIA tests.

There are some significant advantages of using a molecular testing system for Clostridium difficile. Primarily, the results are extremely accurate. Most published studies show that molecular methods are 96-99% sensitive and 97-100% specific, much improved over previous testing methods and previous “gold standards.” See Table 5, Performance of Various Testing Methods of Clostridium difficile. Results are not only accurate, but are available quickly to permit the employment of infectious control isolation procedures and antibiotic therapy. The superior performance of molecular testing for C. difficile should provide confidence in interpreting and reacting to both positive and negative test results. Physicians need not distrust negative test results, and can now spare patients from unnecessary empiric antibiotic treatment and avoid isolation procedures. Real-time polymerase chain reaction (PCR) has the potential to
improve CDI diagnosis because of increased accuracy along with availability of rapid results similar in speed to that of EIAs. Two of the current molecular testing procedures can be adopted in small hospitals or clinic laboratories without the need for purchasing a thermocycler, or the need to have laboratory personnel specially trained using molecular techniques. Those methods are the Cefeid GeneXpert and the Meridian Bioscience Illumigene *C. difficile* test.

One of the major obstacles to adopting PCR is cost. However, laboratory administrators and hospital administrators need to look beyond the financial impact of molecular testing to the lab budget, and look instead to the improvement of the total hospital budget as a result of molecular testing for *C. difficile*. There are an increasing number of reports that support the use of molecular testing upfront to minimize the number of incorrect test results following testing for CDI. It has been argued that the increased costs associated with using molecular tests for all cases of suspected CDI are justified because of the long-term savings related to more accurate diagnosis. For example, providing accurate and rapid results could eliminate the unnecessary use of antibiotics and personal protective equipment and reduce the rate of needless isolation. These cost savings could more than offset the added cost of PCR testing. Further, the CDC believes that an accurate diagnosis and proper treatment could lower mortality from *C. difficile* infection (9).

Current guidelines from the American Society for Microbiology (12) recommend that clinical laboratories use a two-step algorithm which is cost-effective and highly sensitive for the detection of toxigenic *Clostridium difficile*. The first step is using a GDH based system plus an immunoassay such as in C. diff Quick Chek Complete (See Table 4). If both the GDH and the immunoassay are positive, the result can be reported as positive for toxigenic *Clostridium difficile*. If the GDH is positive, but the immunoassay is negative further testing such as molecular testing is necessary to confirm the presence or absence of toxigenic *Clostridium difficile*. See Table 6 for molecular methods.

Data that I obtained from the College of Pathology Q.C. Microbiology Program (CAP) from 2015, showed that very few hospitals were reporting *C. difficile* test results using stand-alone EIA testing and culture cytotoxin assay. Most laboratories were reporting *C. difficile* tests results from either a two-step procedure with GDH and EIA, or in the majority of cases a molecular method.

**G. TREATMENT**

The treatment of *C. difficile*-associated infection depends on its clinical presentation. In otherwise healthy adults, the first step is to discontinue the precipitating antibiotic, if possible, and administer fluids and electrolytes to maintain hydration. With this conservative therapy, diarrhea can be expected to resolve in 15 to 20 percent of patients (6).

Standard antibiotic therapy for *Clostridium difficile*-associated disease is oral vancomycin or oral metronidazole. Metronidazole was often preferred because of its low cost, its perceived high efficacy, and the concerns about vancomycin creating selective pressure favoring development of resistance in other nosocomial pathogens (e.g., enterococci). However, a recent comparison of these antimicrobials found that vancomycin was superior to metronidazole in...
patients with severe CDAI because it resulted in a better therapeutic response and fewer relapses (6).

Newer therapies are being developed to address the population of patients that do not respond well to either vancomycin or metronidazole, or have multiple continued relapses. A new antibiotic approved by the FDA in 2011 for *C. difficile* infection is Dificid (fidaxomicin), which can be administered orally. In one comparison with vancomycin, fidaxomicin showed equal or better results than vancomycin and the rate of recurrence of *C. difficile* was significantly less (10). Other recent studies have shown that the recurrence rate of *C. difficile* in patients who took fidaxomicin was lower than among those who took vancomycin or metronidazole. However, fidaxomicin costs considerably more than metronidazole and vancomycin (9, 12).

An additional new therapy for *C. difficile* infection is the use of a narrow spectrum, non-absorbable antibiotic (such as Nitazoxanide or Rifaximin) that causes minimal changes in the normal intestinal flora. Another potential therapy is the use of non-antibiotic toxin binding agents, such as polymers, which are designed to bind toxin and allow recovery of the normal protective colonic flora, thereby limiting *C. difficile* to its spore form so that no toxin is produced. Another therapeutic approach is to increase the patient’s antibody response to *C. difficile* and its toxins through use of vaccines, such as intravenous immunoglobulin, or monoclonal antibodies that can combat the patient’s disease through passive immunity. The results of trials aimed at improving the patient’s antibody response are varied.

Another recent approach for the treatment of *C. difficile* infection is the use of live bacterial organisms (probiotics) to restore the patient’s normal microbial colonic flora. Commonly used species include *Lactobacillus acidophilus, L. bulgaricus, L. planatarum,* and *Saccharomyces boulardii* alone or in combination. Recent reports by the Cochrane Collaboration Group (An international network of 28,000 individuals from over 100 countries that review evidence-based health care) have shown that probiotics appear to confer protection against *C. difficile* symptoms in people taking antibiotics. The incidence of *C. difficile* diarrhea is statically less in the probiotic receiving group than in the non-probiotic group. Probiotics appears to prevent the symptoms of *C. difficile* infection but do not prevent the infection itself.

Yet another recent approach is fecal bacteriotherapy or fecal transplants to restore the normal intestinal flora. Stool is instilled in the patient from either end of the GI tract (either via an NG tube or an enema). Several case series have documented efficacy rates of 94 to 100% when enteral “stool transplants” were used in patients who had failed multiple courses of conventional therapy. This approach has had good response rates; however, the enemas are difficult to perform, and there is a risk of introducing retroviruses or other infectious agents. Also, most patients feel an innate aversion or reluctance toward this type of treatment. Scientists are looking at the possibility of finding a single bacterium that could be given to the patient—in a capsule, for example—that could restore the normal flora balance in the colon and cure the disease.

A recent novel approach of treating *C. difficile* recurrent infection is with the treatment of human monoclonal antibodies to neutralize *C. difficile* toxin A and toxin B. A group of
investigators at the University of Massachusetts and from Merck Pharmaceuticals developed monoclonal antibodies, which is called bezlotoxumab, to treat recurrent *C. difficile* infection. A single, one-time infusion of bezlotoxumab significantly reduced the recurrence of *C. difficile* infection. Data has been submitted to the FDA in 2013 for approval (12).

Finally, surgery remains an option for treatment of severe CDAI. Total colectomy may be necessary in some severe situations, particularly if initial medical therapy fails, there are multiple relapses, or a perforation or toxic megacolon develops. Although surgical intervention may be necessary in a few rare cases, surgery in CDAI patients has a high mortality rate.

**H. PREVENTION**

Prevention of *C. difficile*-associated infection can be challenging for healthcare facilities because the *C. difficile* organism produces spores that can survive for long periods of time in the environment, the spores are resistant to commonly used disinfectants, and the disease is easily spread from patient to patient. With this in mind, there are three primary methods for preventing *C. difficile* infection in healthcare facilities: 1) hand hygiene and other infection control strategies, 2) thorough environmental decontamination, and 3) antibiotic restriction. The goal of these three measures is to prevent the patient from being colonized by *C. difficile*, reduce the incidence of spores in the environment, and prevent cross-transmission.

The Guidelines for Infection Control in Health Care Facilities published by the CDC note that proper hand hygiene is the single most important factor in protecting patients from *C. difficile* (7). Hand hygiene limits the cross-transmission of *C. difficile* from patient to patient, or from the environment to the patient via the hands of the healthcare workers. The CDC currently recommends that hands must be washed with soap and water when caring for patients with *C. difficile*, as the commonly used alcohol-based hand gel is ineffective against the spores of this organism and may be insufficient to prevent cross-transmission of *C. difficile* spores. Hand washing with soap and water removes spores more efficiently than alcohol-based gels or wipes.

The choice of what type of hand hygiene material to use depends upon whether the healthcare facility is under endemic control or outbreak situation. New CDC guidelines recommend a tiered approach for control: 1) the use of alcohol hand rubs during periods of endemic rates of infection, and 2) the use of soap and water in an outbreak situation. Unfortunately, studies have repeatedly shown that handwashing compliance rates in hospitals using any hand washing method are generally less than 50 percent. Along with hand hygiene, healthcare providers should use good barrier precautions, including gloves and gowns, with *C. difficile*-positive patients, isolating positive patients in a room by themselves if possible, and providing private bathrooms. See Table 7, How to Prevent *C. difficile*-Associated Infection in Healthcare Settings. When proper infection control measures are not practiced, the rate of *C. difficile* infection increases, and the healthcare provider may transmit the organism to others for whom they provide care.
The second primary method for preventing \textit{C. difficile}-associated infection is through environmental decontamination. To limit the spread of \textit{C. difficile}, it is essential that all surfaces and all equipment in the room of a \textit{C. difficile}-positive patient be cleaned thoroughly at the time of patient discharge using a 1:10 dilution of sodium hypochlorite (bleach). Data from the Healthcare Infection Control Practices Advisory Committee (HICPAC) has shown that "meticulous environmental cleaning followed by disinfection using hypochlorite-based germicides reduces the contamination of the patient care environment, results in reduced rates of CDAD, and is successful in reducing patient-to-patient spread of \textit{C. difficile}.” Hospitals that have stepped up efforts to more thoroughly clean hospital wards have effectively controlled the spread of \textit{C. difficile} (7, 11). Of course as with any infection control measure, healthcare worker compliance is the key to success.

Antibiotic restriction is the third method for preventing \textit{C. difficile}-associated infections. Hospitals that restrict the use of the type of antibiotics frequently associated with this infection have had more success in protecting patients and preventing disease. Multiple studies show that the restriction of clindamycin, third generation cephalosporins, and fluoroquinolones in the hospital environment can lead to a significant decrease in nosocomial infections caused by \textit{C. difficile} (6). The goal of the program should be to reduce the use of broad-spectrum antibiotics and increase the use of narrow-spectrum antibiotics. A CDC study in 2014 estimated that a 30 percent reduction in the use of broad-spectrum antibiotics would result in a 26 percent reduction of \textit{C. difficile} infection.

To understand the incidence of infection and to determine whether implemented measures have been adequate to prevent epidemics in healthcare facilities, it is important that healthcare facilities monitor the number of \textit{C. difficile}-associated disease cases. If an increase in rates or severity is observed, healthcare facilities should reassess compliance with the recommended infection control measures for preventing cases of CDAD. Equally important, healthcare facilities should use laboratory test methods that provide for rapid detection of \textit{C. difficile}-positive patients, thereby facilitating earlier appropriate treatment and earlier implementation of infection control interventions to prevent transmission of \textit{C. difficile} to other patients.

I. CASE STUDY

Mr. Smith, a 72-year-old male, was seen in the emergency department of a local community hospital because of a sudden onset of fever, shaking chills, chest pain, shortness of breath, and a rust-colored productive cough. The physician in the emergency department ordered a chest X-ray, CBC, sputum Gram stain and culture. The patient told the physician that one week before coming to the emergency department, he had developed “a bad cold,” but prior to that he had been in good health. A physical examination of the patient revealed a very sick man with a fever of 103°F, irregular pulse of 124 beats per minute, and blood pressure 112/70. His chest X-ray showed a sharply defined homogeneous pulmonary density involving the right middle lobe, consistent with bacterial pneumonia. Other initial laboratory data included a total leukocyte
count of 18,500/μL with 71% segmented neutrophils. A freshly collected sputum specimen was submitted to the laboratory for Gram stain and sputum culture. The Gram stain revealed numerous neutrophils and numerous Gram positive diplococci. He was admitted to ICU with a diagnosis of pneumococcal pneumonia and started on IV ceftriaxone and other supportive measures for pneumonia.

The patient improved after five days in the ICU and was transferred to a general medicine ward with two other patients. He stayed on this unit three days, remained on ceftriaxone and was symptom-free of pneumonia.

All three patients in this general medicine unit had a variety of illnesses and were seen by many different nurses, physicians, physical therapists, respiratory therapists, and laboratory personnel. Mr. Smith never knew what types of illnesses his two other roommates had. He did mention that he noticed that some of the hospital personnel were diligent about hand washing, while others were not. He was discharged to his home feeling well, but within 48 hours he developed severe diarrhea. His wife called her husband’s primary care physician, who ordered Imodium (loperamide), an over-the-counter medicine to treat diarrhea. However, after an additional 24 hours, the patient was getting worse, with profuse watery diarrhea at least 4 times per day, fever, loss of appetite, abdominal cramps, and nausea. His physician re-admitted Mr. Smith to the same hospital in a general medicine ward with one another patient. Mr. Smith’s physical exam revealed that he had severe diarrhea with abdominal pain and cramps, a marked distension of the abdomen, a fever of 102°F, and severe dehydration. A CBC showed a white blood cell count of 19,000/μL.

Other laboratory studies revealed a hemoglobin level of 9.9 g/dL, a platelet count of 92,000/μL, and notable serum chemistries of a BUN of 77 mg/dL and a creatinine of 4.4 mg/dL. His physician ordered a stool culture, an ova and parasite examination, and a C. difficile toxin test. All three stool tests were negative. Because of the patient’s severe diarrhea and his other symptoms, the physician was suspicious of the accuracy of the negative C. difficile test and ordered two more tests for Clostridium difficile. Again, both C. difficile toxin tests were reported as negative. The laboratory used a method that detected C. difficile toxins by enzyme-linked immunoassays (EIA), the most common laboratory test for diagnosing C. difficile–associated infection.

On his third day of hospitalization on the general medicine ward, his primary care physician requested a consultation by an infectious disease specialist since Mr. Smith was not improving. His primary physician wondered if Mr. Smith had acquired food poisoning or might have acquired some other infectious agent, since he believed the negative C. difficile toxin tests ruled out C. difficile infection. The infectious disease physician examined the patient and then requested that a stool sample be sent to a reference laboratory for another type of C. difficile test. The reference laboratory performed molecular testing on the stool sample and within a few hours results were available. Mr. Smith’s C. difficile molecular test was reported as positive. Mr. Smith was immediately transferred to a private room and started on oral metronidazole.
Infection control procedures were initiated in Mr. Smith’s room, including barrier isolation techniques—gloves, gowns, and meticulous hand washing with alcohol wipes (the medical/nursing staff were not aware that soap and water is more effective than alcohol wipes for removing \textit{C. difficile} spores). After three days of oral metronidazole, the patient was not improving. The patient was still having multiple episodes of watery diarrhea, with nausea, fever, chills, abdominal pain, and cramps. His infectious disease physician switched the patient to oral vancomycin. The patient began to improve within 3 days, and the oral vancomycin was continued for the usual 10-day course.

About this time, the infection control personnel at the community hospital noted that the incidence of \textit{C. difficile} infection in their ICU and general medicine units had dramatically increased by threefold. Later, patients in other units of the hospital began experiencing severe diarrhea. Infection control personnel stepped in once they determined the hospital was experiencing an epidemic and immediately implemented CDC guidelines, such as: 1) using contact precautions for patients with known or suspected \textit{C. difficile} infection, 2) placing positive patients, or suspected-positive patients, in private rooms with private bathrooms, 3) performing proper hand hygiene using soap and water, 4) changing gloves and gowns between patients, and 5) ensuring adequate cleaning and disinfection of environmental surfaces with hypochlorite based disinfectant. These procedures were followed throughout the hospital, and after several weeks the incidence of infection of \textit{Clostridium difficile} seemed to be decreasing.

The infectious disease physician recommended to the clinical laboratory that a more sensitive test would improve patient care and help reduce the incidence of infection throughout the hospital. The infectious disease physician also recommended that the hospital begin to report the incidence of \textit{C. difficile} to the California Department of Public Health, a reporting requirement that began in January 2009. The hospital was not aware of this requirement and implemented the new reporting requirement immediately. The infectious disease physician pointed out that the value of the new reporting requirement is that it allows hospitals to compare their rates of \textit{C. difficile} infection to other similar medical facilities. Hospitals can use this information to determine what measures (best practices) are working at other medical facilities to reduce rates of infection, and what additional steps need to be taken to treat and prevent \textit{C. difficile} infections.

Unfortunately, the \textit{C. difficile} saga does not end here for Mr. Smith. Mr. Smith was discharged home after 10 days on oral vancomycin, but 48 hours after stopping his oral vancomycin, his diarrhea symptoms developed again. Mr. Smith had a relapse of \textit{C. difficile} infection, which occurs in about 20% of patients. His symptoms, however, were not severe enough that he needed to be re-admitted. His physician recommended that since his symptoms were minor at this time, he should begin a new course of oral vancomycin for 10 days and perhaps try a course of probiotics. Mr. Smith’s physician wanted to avoid readmitting Mr. Smith to the hospital so that he would not transmit \textit{C. difficile} spores to other patients and would not potentially re-infect himself from the contaminated hospital environment. Mr. Smith did improve and was eventually clear of symptoms.
Discussion of Case Study

This case study shows the typical course of infection and disease progression in patients who acquire *C. difficile* spores in a healthcare setting. Generally, the patient is admitted to the hospital for one illness, and while in the hospital acquires the spores of *C. difficile* and develops a *C. difficile* infection. Symptoms usually begin within 4-10 days of admission, but they may not develop until a few weeks after the person stops taking the antibiotic prescribed for their initial complaint upon admission. Normal intestinal flora helps prevent many invading microbial diseases from establishing in the colon and causing disease. In this case study, the patient was taking ceftriaxone, a third generation broad spectrum cephalosporin that can drastically alter the normal bowel flora. The major risk factors for Mr. Smith for acquiring *C. difficile* infection were: 1) antibiotic therapy, 2) age >65 years old, 3) hospitalization, 4) being cared for in an ICU setting, and 5) prolonged hospitalization.

The main factor affecting the course of Mr. Smith’s disease was his physician’s failure to recognize that Mr. Smith acquired the spores of *C. difficile* while in the hospital, but did not develop the disease and symptoms of *C. difficile* infection until he went home sometime later. It is not uncommon for patients to develop symptoms a week or two after the individual stops taking an antibiotic. Once Mr. Smith developed severe diarrhea and other symptoms consistent with *C. difficile* infection, his physician should have been able to access information from the patient or his medical chart to show that he was recently discharged from a hospital. The physician therefore would have not ordered Imodium, and would have immediately admitted him to a hospital into a private room until his diagnosis was determined. This measure would have prevented contamination of healthcare workers, the healthcare environment, and other patients.

The critical issue in this case study was the clinical laboratory’s use of an insensitive toxin test for *C. difficile*. Studies show that many EIA toxin test systems have a poor sensitivity, ranging from 33% to 80%, which means that about 20-60% false-negative results can occur. Currently, 80% of U.S. hospitals use some type of EIA toxin test because of their low cost, rapid turnaround time, and ease of use. However, the CDC (9) has recently recommended molecular testing, which provides the most sensitive and accurate testing for *C. difficile* infection. As a consequence of the poor sensitivity of the *C. difficile* EIA toxin test, Mr. Smith was placed in a general medicine ward and not in isolation using appropriate barrier precautions. Therefore, Mr. Smith spread the spores of *C. difficile* to the medical staff, the environment, and to other patients. His primary physician was not aware of the poor sensitivity of many toxin EIA tests, so the diagnosis and private room placement were delayed. The health care workers who cared for Mr. Smith during his stay in the general medicine unit unfortunately washed their hands (if at all) with alcohol wipes rather than using soap and water. Consequently, health care workers were not effectively removing *C. difficile* spores from their hands, and they transmitted the spores to other health care workers and to other patients. Many other recent studies show that nurses, physicians and other hospital personnel fail to wash their hands using any type of technique before and after each patient.
In this particular case study, oral vancomycin seemed to be more effective in treating Mr. Smith’s *C. difficile* infection than oral metronidazole. However, he relapsed after the treatment was stopped. Vancomycin can result in a better therapeutic response and fewer relapses, but that was not initially the case for Mr. Smith. Not returning to the hospital after his relapse prevented further spread of the pathogen. Although results from studies treating *C. difficile* infection using probiotics is mixed and with no clear advantage for their usage, Mr. Smith eventually improved, although the exact reason is not known.

This case study illustrates the significant financial impact of *C. difficile* infection to Mr. Smith, the community hospital, and the U.S. healthcare system in general. Mr. Smith, most likely a Medicare patient, probably stayed an additional 13 days in the hospital because of *C. difficile* infection acquired during his hospitalization. Some of these days were spent in a private room under contact precautions, substantially increasing his overall hospital costs.

It is unknown whether the *C. difficile* Mr. Smith acquired was the new hyper-virulent *C. difficile* strain of NAP1/BI/027. Most laboratories do not isolate the organism, so the California Department of Public Health (CDPH) may not be able to perform molecular typing on isolates. In major epidemics, however, the CDPH may request that the laboratory isolate the organism so that molecular typing can be performed.

This case and accompanying discussion highlight the need for efforts to prevent *Clostridium difficile* infection in healthcare facilities through the judicious use of antibiotics and current infection control recommendations. The early recognition and accurate determination of *C. difficile* by using sensitive laboratory tests is essential to reduce the incidence of infection.

**J. CONCLUSIONS**

The recent emergence of the *C. difficile* strain NAP1/BI/027 has illustrated how suddenly a disease can change because of alterations in the genetic makeup of an organism, leading to dramatic increases in pathogenicity and thus morbidity and mortality. The new 027 strain of *C. difficile* has significant pathogenic mechanisms, including producing hyper-amounts of toxin A and B, producing a binary toxin, an increased sporulation rate, an increased ability to adhere to the lining of the colon, and resistance to the fluoroquinolone group of antibiotics.

Whereas in the past *C. difficile* infection was thought of as a “nuisance” and easily treated, it now represents a severe disease for hospitalized patients. Data from the CDC show that the incidence, the mortality, and the morbidity is much higher than most healthcare providers previously believed, and that all three have actually been increasing since 2002. Further, CDAI represents a significant expense to the healthcare system, causing more illness, longer hospitalizations, and sometimes death. The recent emergence of severe CDAI has forced changes in laboratory diagnosis, patient care, and infection control.

One necessary change is a rapid, sensitive laboratory test to permit prompt diagnosis of patients with CDAI. Current stand-alone EIA toxin tests lack the sensitivity required to detect CDAI in patients. Therefore, it is recommended by the American Society for Microbiology (12) that clinical laboratories use a two-step algorithm employing an assay with GDH and an
immunoassay for initial testing of samples. If both tests are positive, the *Clostridium difficile* produces toxin. If, however, the GDH is positive and the immunoassay is negative an additional testing method such as a molecular method must be used to verify the production of toxin.

Healthcare facilities also need to improve their hand washing, infection control, and antibiotic therapy protocols. Outbreaks in healthcare facilities can be challenging because of the nature of the *C. difficile* organism, with its ability to survive for long periods of time in the environment, its resistance to common disinfectants, its ability to easily spread from patient to patient, and its opportunism in patients who are receiving or have received antibiotic therapy. However, improvements in the incidence of infection have been seen in those hospitals that have implemented the following protocols: 1) wash hands with soap and water instead of using alcohol-based hand rubs when caring for *C. difficile*-positive patients, 2) disinfect *C. difficile* positive patient rooms with bleach, and 3) control the use of the three known antibiotic causes of *C. difficile* infection: clindamycin, third generation cephalosporins, and fluoroquinolones.

California’s Department of Public Health (CDPH) new reporting requirement that became available in January 2011 shows the incidence of *C. difficile* infection in hospitals and clinics. See Table 2. Hospital-Onset *Clostridium difficile* Infections Reported by California Hospitals by Year (2010-2014) (14). Although the CDPH states in their first public health surveillance report on *C. difficile* infection that the data is not perfect and more work needs to be done concerning the quality and its completeness of the report, it is a step in the right direction to assess how hospitals are managing to control the spread of infection. Further, as more of the public becomes aware of the incidence of *C. difficile* infection, this will force changes in the healthcare environment, perhaps including improved compliance with hand washing protocols, barrier precautions, and judicious antibiotic use. Also, the new state requirement may give hospitals a better idea of what they may be doing right or wrong to prevent disease—particularly when their rate of infection is compared with that of other competing hospitals.

There is no doubt that *Clostridium difficile* infection is on the rise, and the new *C. difficile* 027 strain is particularly vexing. However, laboratory personnel and healthcare providers who understand and implement the most recent diagnostic, treatment, and prevention protocols can substantially reduce the incidence of *Clostridium difficile*-associated infection. It is imperative that physicians, infection control specialists, and laboratory personnel appreciate the pros and cons of the various diagnostic tests for *C. difficile*. Low sensitivity of an assay will lead to missed diagnoses. This can be detrimental to the patient, by delaying appropriate treatment. It also can be detrimental to the hospital, because often patients are not isolated or are inappropriately removed from isolation after a false negative test result—which can lead to further spread and more cases of CDAI. Additionally, low specificity results in more false-positive cases in patients who may subsequently have necessary antibiotics curtailed and receive unnecessary treatment for CDAI (11,12).
K. REFERENCES
Table 1. Risk Factors for *Clostridium difficile*-Associated Infection

- Admission to hospital or other healthcare facility
- Antibiotic therapy
- Age >65 years
- Admission to intensive care unit
- Immunosuppressive therapy
- Multiple and severe underlying diseases
- Placement of nasogastric tube
- Prolonged hospital stay
- Recent surgical procedure
- Residing in a nursing home
- Sharing a hospital room with a *C. difficile*-infected patient
- Use of antacids or medicine that alter the pH of the bowel

Table 2. Hospital-Onset *Clostridium difficile* Infections Reported by California Hospitals by Year (2010–14)*

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Hospital Onset Cases</th>
<th>Number of Hospitals Reporting Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010**</td>
<td>9870</td>
<td>350</td>
</tr>
<tr>
<td>2011</td>
<td>10938</td>
<td>378</td>
</tr>
<tr>
<td>2012</td>
<td>10667</td>
<td>388</td>
</tr>
<tr>
<td>2013</td>
<td>10685</td>
<td>384</td>
</tr>
<tr>
<td>2014</td>
<td>10688</td>
<td>392</td>
</tr>
</tbody>
</table>

** Only partial year (10 months) reported

Table 3. Common Symptoms and Diseases Associated with *C. difficile* Infection

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>frequent, watery diarrhea</td>
<td>diarrhea</td>
</tr>
<tr>
<td>abdominal pain and cramps</td>
<td>pseudomembranous colitis</td>
</tr>
<tr>
<td>fever</td>
<td>toxic megacolon</td>
</tr>
<tr>
<td>pus in stool</td>
<td>perforation of the colon</td>
</tr>
<tr>
<td>bloody stool</td>
<td>sepsis</td>
</tr>
<tr>
<td>nausea</td>
<td>death (rarely)</td>
</tr>
<tr>
<td>dehydration</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4. Methods and Tests for the Detection of \textit{C. difficile}

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Available tests and sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Culture cytotoxicity assay for Toxin B</td>
<td>65-80% sensitive, 96-98% specific</td>
<td>Requires 24-48 hr. Need tissue cells and special equipment</td>
<td>\textit{C. difficile} Tox B Test (TechLab, Inc., Blacksburg, VA) Bartels Cytotoxicity Assay (Trinity Biotech Co., Carlsbad, CA)</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>70% sensitive, but does not detect toxin</td>
<td>Requires specialized media and anaerobic techniques</td>
<td>CCFA medium (cycloserine-cefoxitin-fructose agar). (Anaerobe Systems, Morgan Hill, CA; Hardy Diagnostics, Santa Maria, CA; Remel, Lenexa, KS)</td>
</tr>
<tr>
<td>Chromogenic agar</td>
<td>Does not detect toxin</td>
<td>Requires anaerobic techniques and at least 24-hour growth</td>
<td>CHROMagar \textit{C. difficile} (bioMerieux Vitek, Hazelwood, MO)</td>
</tr>
</tbody>
</table>

**Tests for antigen**

- Latex agglutination: Rapid, simple. Not extremely sensitive, but has specificity of 98-99%.
  
  Available tests:
  - \textit{C. difficile} Test Kit (Oxoid, Inc., Ogdensburg, NY)

- EIA: Rapid, simple. Detects all \textit{C. difficile} regardless of toxin production.
  
  Available tests:
  - ImmunoCard \textit{C. difficile} (Meridian Biosciences, Inc., Cinn, OH)
  - \textit{C. difficile} QuikChek Toxin Test (TechLab, Inc., Blacksburg, VA)

**Tests for \textit{C. difficile} toxins**

  
  Available tests:
  - \textit{C. difficile} Toxin A (Oxoid, Inc., Ogdensburg, NY)
  - Premier \textit{C. difficile} Tox A (Meridian Diagnostics, Inc., Cinn, OH)
  - Prospect II \textit{C. difficile} Toxin A (Remel, Lenexa, KS)
  - CD Test EIA for Toxin A (BD Diagnostics, Sparks, MD)
  - Triage \textit{C. difficile} Panel (GDH antigen & Toxin A) (Biosite Diagnostics, San Diego, CA)
  - VIDAS CDA 2 Assay EIA (bioMerieux Vitek, Hazelwood, MO)
  - ImmunoCard STAT Toxin A (Meridian Diagnostics, Inc., Cinn, OH)
  - \textit{C. difficile} Toxin A Test Kit (Remel, Lenexa, KS)

  Prospects:
  - Tox A/B QuikChek (TechLab, Inc., Blacksburg, VA)
  - XMpec Clostridium difficile Toxin Test A/B (Oxoid, Inc., Ogdensburg, NY)
  - Tox A/B QuikChek (TechLab, Inc., Blacksburg, VA)
  - Prospect II \textit{C. difficile} Toxin A and B (Remel, Lenexa, KS)
  - VIDAS CDAB Assay (bioMerieux Vitek, Hazelwood, MO)
  - \textit{C. diff} QuikChek Complete (GDH antigen and Toxin A & B) (TechLab, Blacksburg, VA)

- Toxin A & B: Rapid, simple. Some kits have better sensitivity than others.
  
  Available tests:
  - \textit{C. difficile} TOX A/B II (Wampole Labs, Cranberry, NJ; TechLab, Inc., Blacksburg, VA)
  - Premier A and B EIA (Meridian Diagnostics, Inc., Cinn, OH)
  - ImmunoCard Toxins A and B (Meridian Biosciences, Inc., Cinn, OH)
  - XMpec \textit{Clostridium difficile} Toxin Test A/B (Oxoid, Inc., Ogdensburg, NY)
  - Tox A/B QuikChek (TechLab, Inc., Blacksburg, VA)
  - Prospect II \textit{C. difficile} Toxin A and B (Remel, Lenexa, KS)
  - VIDAS CDAB Assay (bioMerieux Vitek, Hazelwood, MO)
  - \textit{C. diff} QuikChek Complete (GDH antigen and Toxin A & B) (TechLab, Blacksburg, VA)

  Prospects:
  - BD GeneOhm C. diff Assay (BD Diagnostics, Sparks, MD)
  - BD Max C. diff Assay (BD Diagnostics, Sparks, MD)
  - Cefiex XMpec Assay (Cefiex, Sunnyvale, CA)
  - Prodesse ProGastro Cd Assay (GenProbe, San Diego, CA)
  - Illumigen \textit{C. difficile} Assay (Meridian Biosciences, Cincinnati, OH)
  - Portrait Toxigenic CD Assay (Great Basin Corporation, West Valley City, UT)
  - Quidel Molecular Direct \textit{C. difficile} Assay (Quidel Corp, San Diego, CA)
  - Verigene \textit{C. difficile} Test (Nanosphere, Northbrook, IL)

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Table 5. Performance of Various Testing Methods for *Clostridium difficile*

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)**</th>
<th>Specificity (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial culture</td>
<td>63-73</td>
<td>85-90</td>
</tr>
<tr>
<td>Cytotoxin assay (CTA)</td>
<td>65-80</td>
<td>96-98</td>
</tr>
<tr>
<td>EIA toxin assays</td>
<td>33-80</td>
<td>83-90</td>
</tr>
<tr>
<td>GDH testing paired with toxin testing</td>
<td>55-85</td>
<td>96-98</td>
</tr>
<tr>
<td>Molecular methods (PCR and other)</td>
<td>96-99</td>
<td>97-100</td>
</tr>
</tbody>
</table>

*Data adapted from reference # 9 and #12.

** Sensitivity, the proportion of positives which are correctly identified.

***Specificity, the proportion of negatives which are correctly identified as negative.
### Table 6. Molecular Tests for the Presence of Toxin-Producing *Clostridium difficile* *

<table>
<thead>
<tr>
<th>Test/Manufacturer</th>
<th>Method</th>
<th>Comments/TAT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Gene Ohm</td>
<td>Uses glass bead lysis. Real-time PCR**</td>
<td>About 1 hour 45 minutes from sample to result.</td>
</tr>
<tr>
<td>C. diff</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.bdbiosciences.com">www.bdbiosciences.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD Max C. diff Assay</td>
<td>Fully automated detection of toxin B gene of <em>C. difficile</em></td>
<td>Results available in less than 3 hours.</td>
</tr>
<tr>
<td>Becton Dickinson</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.bdbiosciences.com">www.bdbiosciences.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefeid GeneXpert Assay</td>
<td>Uses a cartridge based kit Real-time PCR. Detects toxin A, B and CDT¹.</td>
<td>Cartridge is tailored for small hospitals or small labs. Reporting time is 45 minutes.</td>
</tr>
<tr>
<td><a href="http://www.cepheid.com">www.cepheid.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prodesse ProGastro Cd Assay</td>
<td>Uses external extraction Detects toxin B</td>
<td>Takes about 3 hours from sample to result.</td>
</tr>
<tr>
<td><a href="http://www.biomerieux">www.biomerieux</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illumigene <em>C. difficile</em></td>
<td>Uses LAMP technology*** Detects toxin A</td>
<td>Temperature does not need to be raised and lowered. Requires no costly equipment. Reporting time is 1 hour.</td>
</tr>
<tr>
<td>Meridian Bioscience</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.meridianbioscience.com">www.meridianbioscience.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portrait Toxigenic Cd Assay</td>
<td>Uses helicase dependent amplification (HDA)**** Detects toxin B</td>
<td>Isothermal amplification. Requires no costly equipment. Reporting time is 90 min.</td>
</tr>
<tr>
<td>Great Basin Corporation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.gbscience.com">www.gbscience.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quidel Molecular Direct <em>C. difficile</em> Assay</td>
<td>Real-time PCR. Detection of toxin A and toxin B gene</td>
<td>Results available in less than 70 minutes.</td>
</tr>
<tr>
<td><a href="http://www.quidel.com">www.quidel.com</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verigene <em>C. difficile</em> Test</td>
<td>Uses HDA****. Detection of toxin A and B, and CDT¹</td>
<td>Results available in 2 hours</td>
</tr>
<tr>
<td>Nanosphere, Inc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="http://www.nanosphere.us.com">www.nanosphere.us.com</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Data adapted from reference #9 and #12.

¹ CDT. *Clostridium difficile* toxin, or binary toxin. Associated with the 027 ribotype strain of *C. difficile*.

*TAT, turn-around time

**Real-time PCR. Amplification and simultaneous quantifying of a targeted DNA molecule. Permits detection in real time. Much faster than normal PCR techniques.

***LAMP (Loop mediated isothermal amplification technology). Amplification in single step with samples and primers using isothermal (constant temperature) amplification. Method does not require thermal cycler.

****HDA (Helicase dependent amplification). Method of DNA amplification using constant temperature not requiring a thermal cycler. Provides a rapid and inexpensive testing method.
Table 7. How to Prevent *C. difficile*-Associated Infection in Healthcare Settings

1. Use a rapid and accurate diagnostic laboratory test to report positive patients early.

2. Use antibiotics judiciously. Limit the use of clindamycin, third generation cephalosporins, and fluoroquinolones.

3. Use contact precautions for patients with known or suspected *C. difficile*-associated infection.

4. Place positive patients in private rooms. If private rooms are not available, these patients can be placed in rooms with other patients with *C. difficile*-associated infection.

5. Perform proper hand hygiene using either an alcohol-based hand rub or soap and water. If your institution experiences an outbreak, consider using only soap and water for hand hygiene when caring for patients with *C. difficile*-associated infection; alcohol-based hand rubs are not as effective against spore-forming bacteria.

6. Use gloves when entering patients’ rooms and during patient care. Use gowns if soiling of clothes is likely.

7. Implement an environmental cleaning and disinfection strategy: Ensure adequate cleaning and disinfection of environmental surfaces and reusable devices, especially items likely to be contaminated with feces and surfaces that are touched frequently.

8. Use an Environmental Protection Agency (EPA)-registered hypochlorite-based disinfectant for environmental surface disinfection after cleaning in accordance with label instructions.

Adapted from: The Centers for Disease Control and Prevention. 2014. Guidelines for Environmental Control of *Clostridium difficile*. Reference #2, #4, and #7.
REVIEW QUESTIONS
Course #DL-990
Choose the one best answer.

1. Which of the following has not been associated with toxigenic strains of *C. difficile*:
   a. pseudomembranous colitis
   b. toxic megacolon
   c. diarrhea
   d. toxic shock syndrome

2. The most basic precipitating event causing *C. difficile* disease is:
   a. alteration of the normal colonic flora
   b. environmental threshold is exceeded
   c. dehydration
   d. bacterial contamination of drinking water

3. Which of the following is not a risk factor for acquiring *C. difficile*:
   a. hospitalization
   b. age
   c. bacteriuria
   d. antibiotic usage

4. Pathogenesis of *C. difficile* infection primarily involves:
   a. the action of toxin A and toxin B
   b. enzymatic activity of glutamate dehydrogenase
   c. endotoxin activity
   d. cytogenic activation of enterotoxins

5. The emergence of *C. difficile* NAP1/BI/027 has not been associated with:
   a. major epidemics
   b. increased morbidity
   c. increased mortality
   d. increased secondary soft tissue infections

6. An antibiotic generally associated with inducing *C. difficile* infection is:
   a. metronidazole
   b. clindamycin
   c. vancomycin
   d. gentamicin
7. The new epidemic strain of *C. difficile* has which one of the following characteristics:
   a. produces hyper amounts of delta toxin
   b. increased spore size
   c. produces hyper amounts of toxin A and B
   d. increased endotoxin production

8. The new strain of *C. difficile* has been associated with:
   a. an increased mortality and an increased morbidity
   b. an increase in secondary infections and an increased incidence
   c. an increase in secondary infections and a decrease in nosocomial infections
   d. a decreased morbidity and a decreased mortality

9. One potential source of *C. difficile* infections in the out-patient population has been:
   a. poultry
   b. dairy calves
   c. raw spinach
   d. undercooked hamburger

10. The transmission of *C. difficile* is through:
    a. an increase in flagellar motility
    b. the production of toxins
    c. the production of spores
    d. the production of capsules

11. To kill spores of *C. difficile* you need to use:
    a. alcohol
    b. bleach
    c. glutaraldehyde
    d. radiation

12. The best method of preventing *C. difficile* infection in a healthcare setting is:
    a. limiting visitors to ICU
    b. environmental decontamination with alcohol
    c. proper hand hygiene
    d. vaccination

13. The primary symptoms of *C. difficile* infection include:
    a. hypotension, constipation, dehydration
    b. watery diarrhea, fever, bloody stool
    c. dehydration, hypertension, urinary tract infections
d. hemolytic anemia, renal failure, hypotension

14. Three main risk factors for acquiring *C. difficile* infection are:
   a. hospitalization, receiving antibiotics, age >65 years old
   b. receiving analgesics, 40-45 years old, genetic risk factors
   c. prior surgery, 20-35 years old, contaminated water supply
   d. poor diet, age >65 years old, genetic risk factors

15. Normal intestinal bacterial flora help prevent:
   a. invading toxins from adhering to bacterial cells
   b. the production of vegetative cells into spores
   c. invading microbial diseases from establishing in the colon
   d. spores producing toxins

16. The role of *C. difficile* toxin B is:
   a. an enteropathogenic toxin
   b. a potent cytotoxin
   c. activating phagocytosis
   d. inducing tumor necrosis factor

17. Both *C. difficile* toxin A and toxin B cause:
   a. a leukocyte chemotaxis
   b. a decrease in cytokines
   c. a decrease in interleukin factors
   d. a decrease in mucosal permeability

18. The level of serum IgG to *C. difficile* infection determines:
   a. the host response to other clostridial infections
   b. that a high level indicates recurrent disease
   c. that a low level protects asymptomatic carriers
   d. the outcome of infection

19. The role of binary toxin in the *C. difficile* 027 strain is:
   a. to produce two additional toxins
   b. an additional virulence factor
   c. to neutralize toxin A and toxin B
   d. to induce binary bacterial metabolism

20. A potential role of the increased spore rate of the *C. difficile* 027 strain is:
   a. increases organism survival and spread
b. produces more GDH  
c. produces more toxin  
d. increases the growth rate of the organism

21. The function of increased adherence to human intestinal epithelial cells is:  
   a. produces spores  
   b. binds toxin  
   c. establishment of disease  
   d. interferes with EIA testing

22. A particular characteristic of *C. difficile* 027 is:  
   a. resistant to metronidazole  
   b. resistant to vancomycin  
   c. spores are susceptible to alcohol  
   d. resistant to fluoroquinolones

23. Which is not a method for the laboratory diagnosis of *C. difficile* infection:  
   a. tissue culture cytotoxicity assay  
   b. bacterial culture on CCFA media  
   c. endotoxin assay by EIA  
   d. EIA or latex methods for GDH antigen

24. The composition of CCFA used to isolate *C. difficile* from stool is:  
   a. cycloserine-cefoxitin-fructose agar  
   b. cyclomide-cephalosporin-gluconazole agar  
   c. clindamycin-cephalosporin-fluctosine agar  
   d. cycloserine-cephalosporin-fructose agar

25. The major characteristics of *C. difficle* on CCFRA are:  
   a. white to pink colonial appearance, grape-like odor, and fluoresces red under UV light  
   b. yellowish with ground glass colonial appearance, horse manure odor, and fluoresces chartreuse under UV light  
   c. hemolytic with ground glass colonial appearance, no odor, and fluoresces orange under UV light  
   d. yellowish to clear colonial appearance, chicken manure odor, and fluoresces red under UV light

26. Which is not a characteristic of EIA toxin assay testing for *C. difficile*:  
   a. specificity of 83-90%  
   b. sensitivity of >99%
c. low cost

d. rapid turnaround time

27. Which is the correct characteristic regarding GDH antigen testing for *C. difficile*:
   a. sensitivity of >99%
   b. test results generally available in less than 1 hr.
   c. discriminates between toxinogenic and nontoxinogenic isolates
   d. detects the enzyme glycerol dehydrogenase

28. Which of the following characteristics best describes molecular testing for *C. difficile*:
   a. high sensitivity, low specificity
   b. high sensitivity, results are available in 48-72 hr.
   c. high sensitivity, results are available in 1-3 hr.
   d. low cost, detects toxin gene

29. Which is not a treatment of *C. difficile* infection:
   a. oral metronidazole
   b. probiotics
   c. fecal transplant
   d. third generation cephalosporins

30. The Centers for Disease Control and Prevention (CDC) recommends a tiered approach to hand washing in healthcare facilities that consists of:
   a. using soap and water for patients known to have *C. difficile*
   b. using alcohol based hand rubs for patients known to have *C. difficile*
   c. using alcohol based hand rubs followed by soap and water on *C. difficile* negative patients
   d. using only soap and water for all patients followed by wearing gloves