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Clostridium difficile: Biology, Disease, & Treatment

by

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Abstract

Clostridium difficile is a bacterium that under certain conditions can be pathogenic and cause Clostridium difficile Associated Disease (CDAD). Its virulence factors help this bacterium to survive in undesirable environments and spread. Many people unknowingly carry this bacterium in their gastrointestinal tract, yet do not outwardly exhibit disease because normal microbial flora inhibits the growth of this bacterium. Those negatively affected by this bacterium are commonly the elderly and people on broad-spectrum antibiotics. However, an increase in infection is occurring outside of these common victims suggesting that adaptation of the bacterium is allowing it to spread to other populations. Prevention is still the number one priority in containing this bacterium as it can survive in an infective form virtually anywhere due to its ability to form endospores. It has also become resistant to antibiotics that had previously cured infections caused by *C. difficile*. An increasing amount of research is being conducted to find alternative treatments for those affected with CDAD. One alternative method that looks to be promising is fecal implantation, but more research needs to be conducted before it can be implemented as a standard treatment.

Biology

Clostridium difficile is a large rod-shaped bacterium that can form spores. The endospore structure of *C. difficile* allows this organism to withstand harsh environments and remain viable for extended periods. The cell is not able to divide and replicate in this state because it is conserving its metabolic capacity and energy storage until environmental conditions are optimal. An endospore structure typically includes an exosporium, cortex, and a core. The exosporium is a covering around the spore which consists of thick layers of protein (Lawley *et al*., 2009). The cortex beneath the exosporium is composed of thick peptidoglycan. The core contains the nucleoid and the ribosomes. Dipicolinic acid located within the center of the spore is incorporated with calcium ions and helps protect the cell from heat. The calcium ions within the dipicolinic acid protect the core against wet and dry heat, as well as oxidizing agents. Small acid-soluble, DNA-binding proteins attach to the DNA within the spore and protect it from heat, radiation, desiccation, and chemicals. Another mechanism used to protect the cell from heat and radiation is removing water from the core of the cell. Since *C. difficile* is an obligate anaerobic bacterium, the endospore also protects the bacterium from oxygen (Edwards, Suarez, & McBride, 2013). Without the endospore, the cell would suffer from cell damage when exposed to oxygen due to the build-up of superoxide and hydrogen peroxide which the organism cannot remove (Imlay, 2003). Once the bacterium is not under harsh conditions, it will lose the endospore and begins to germinate again with the assistance of DNA repair enzymes (Marquis, Sim, and Shin, 2008). The composition of the endospore aides in protecting the vegetative cell and allows it to survive for an extended period of time without nutrients.

This bacterium is heterotrophic, meaning it cannot produce its energy source and needs an outside carbon and energy source for its survival. It utilizes fermentative metabolism, which

allows it to utilize sugars and amino acids without the need for oxygen. It oxidizes amino acids by using the Stickland reaction, and its toxin production has been thought to rely on available amino acids, with preferences shown to be proline. (Bouillaut *et al*., 2013) A human's microbiome assists in the breakdown of complex macronutrients into their simpler forms to allow the body to utilize the main component of the compounds. *C. difficile* will also use these compounds to obtain its nutrient and energy needs to survive in the gastrointestinal tract. Amino acid fermentation uses essential amino acids as the source of energy. Since we require essential amino acids and we get them through our diet, the gastrointestinal tract where *C. difficile* resides, allows it to be perfectly positioned to benefit from our own ingested food.

Virulence Factors

Specific innate immune responses can inhibit bacterial infection. However, many bacteria can adapt and bypass these mechanisms to continue infecting the individual. The production of the mucus layer in the gastrointestinal tract acts as a barrier to prevent antigen attachment to host cells. *C. difficile*, among other bacteria, have developed mechanisms to overcome this barrier and attach to host cells (Hecht, Pothoulakis, LaMont, & Madara. 1988). *C. difficile* can bypass gastric acid production due to endospore formation. The production of bile salt by the gall bladder typically disrupts the cell membrane of most vegetative bacteria leading to cell death. *C. difficile* endospores resist the action of bile salts and bile salts also act to trigger endospores to become vegetative cell (Sorg & Sonenshein, 2008).

C. difficile needs to attach to the host cell surface to avoid being washed away with the digested food products. The bacterium first needs to be able to differentiate their specific host cells from other cells in the gastrointestinal tract. New strains of *C. difficile* demonstrate that microbial flora cells do not always affect the invasion of *C. difficile* bacterium. The use of a

variety of recognition proteins assists the bacterium in finding the host cell. The mechanisms involved with attachment to host cells include the use of adhesins, in particular, the product of gene cwp66. Another protein that the bacterium produces called the phase-variable cell wall protein CmpV helps the bacterium in immune evasion once it is attached to the host cell (Janoir, 2016).

C. difficile only produces disease when it returns to its vegetative form in the gastrointestinal tract and is allowed to produce toxins. In particular, most pathogenic strains produce toxin A, (TcdA) and toxin B (TcdB). Collectively these toxins catalyze a covalent modification which affect actin, which is critical in cell functions (Popoff, 2014). More specifically these toxins function by catalyzing the monoglucosylation of a threonine residue on Rho family proteins, leading to Rho inactivation (Janoir, 2016). Rho inactivation then leads to actin re-organization which affects the cell cytoskeleton and ultimately leads to cell death. This mechanism benefits the bacterium by gaining access to more nutrients while also escaping the immune responses like phagocytosis (Popoff, 2014).

Symptoms of CDAD can be caused by either toxin A, toxin B, or a combination of both (Lyerly, Krivan, & Wilkins, 1988). Some strains of *C. difficile* (e.g., NADP1/027) demonstrate increased expression of A and B toxins due to alterations in the regulation of toxin production (Blossom & McDonald, 2007). Other strains produce a binary toxin, referred to as CDT that has not yet been determined to have any effect on animal models studied. It is composed of two proteins, CDTa and CDTb, which express ADP-ribosyltransferase activity, but it is not cytotoxic. Strains that lack toxin A and B, but still produce binary toxin are not pathogenic (Popoff, Rubin, Gill, & Boquet, 1988).

One study was able to find a strain of the bacterium that only produced toxin B. This is particularly dangerous for humans because normally enzyme immunoassays to help detect and confirm disease caused by *C. difficile* only recognize toxin A (Binning et al., 1994). In vitro testing suggests that toxin B can produce more toxicity than toxin A, but this has not been observed in humans yet (Drudy, Fanning, & Kyne, 2007). Only one pathogenic toxinotype of C. difficile that expresses only the B toxin (type 8) has been linked to C. difficile outbreaks (Drudy, Fanning, & Kyne, 2007). There have only been few occurrences within the last thirty years of toxinotype 8 *C. difficile* causing disease (Drudy, Fanning, & Kyne, 2007).

Diagnosis of CDAD

Many tests can be performed in the laboratory to test for CDAD. A person will typically need to indicate a presence of the symptoms, and then appropriate testing can be determined. It is often difficult to differentiate this infection from other bacterial infections as many of the symptoms and signs are similar. Examples of some bacterial infections that also cause diarrhea and stomach cramps include enteric *Salmonella typhi* (Crump, Sjolund-Karlsson, Gordon, & Parry, 2015). Enterohemorrhagic *Escherichia coli* also causes stomach cramps and bloody diarrhea which can occur in severe CDAD (Palmer & Skaar, 2019). Treatments vary greatly, so laboratory tests are necessary to provide proper care. Testing includes bacteria culture, toxigenic culture, cell culture neutralization assay, glutamate dehydrogenase assay, toxin enzyme immunoassay, and nucleic acid amplification. Not all tests are necessary for determining the presence of CDAD. The availability of testing methods is a factor for deciding which to use, especially in rural areas. In the United States, the preferred method of diagnosis is nucleic acid amplification testing (Peng *et al.* 2018). When compared to the other options this method is more specific and has higher sensitivity while also providing quicker results. If this method of diagnosing the patient is not

available, a combination of testing methods is necessary (Burke and Lamont, 2014). Glutamate dehydrogenase assay followed by the immunotesting for toxins A and B are used but have a lower sensitivity than nucleic acid amplification tests (Boyanton *et al.* 2012). With the rise of virulent strains, some with just one of the toxins, it is essential to now include both toxins when using the latter testing method. If necessary, a colonoscopy can be performed which can also help to see the extent of damage done by the overgrowth of the bacterium. A small camera in the form of a tube is inserted into the anus and pushed up into the colon to see the inside of the large intestine. If *C. difficile* is present, it will be pronounced as yellow looking patches will be seen on the colon wall where the bacteria is growing.

Epidemiology of CDAD

The disease mainly occurs in hospitalized patients infected with *C. difficile* undergoing antibiotic therapy that disrupts the normal microflora of the gastrointestinal tract allowing for the overgrowth of *C. difficile* and toxin production (Heinlen & Ballard, 2011). CDAD can also appear in patients having gastrointestinal tract surgery or displaying a reduction in gastric acid production due to medication. CDAD is starting to appear in patients within the community at large who are not on antimicrobial therapy (Halvorson, Cedfeldt & Hunter, 2011).

Through the formation of an endospore, *C. difficile* can live on fomites or inanimate objects. As previously stated, the spores can resist most decontamination efforts, so special care is needed when cleaning hospital rooms. The fecal-oral route spreads *C. difficile*. If a person colonized by *C. difficile* does not properly wash their hands after defecating, they have the potential to spread the bacterium through the spores that exited the body with the bowel movement.

For many years, the demographics of people affected by the invasion of *C. difficile* were elderly patients and people taking broad-spectrum antibiotics. CDAD is more prevalent in hospitals and nursing homes and is classified as a nosocomial infection. Some regions of the United States like the southeast have seen CDAD surpass MRSA as the number one most common infection associated with healthcare facilities (Miller et al. 2011). However, a broader demographic of the population is starting to become affected by overgrowth of this bacterium. A rising number of younger patients as well as people who were not previously exposed to healthcare settings are becoming affected by overgrowth of *C. difficile*. Scientists believe this is due to the rise of a new virulent strain that can withstand the human body's standard defense mechanisms (McDonald *et al.*, 2005).

A study conducted in 2015 observed ten geographic regions to determine the presence of CDAD and its relevance among different demographics of the population. The data collected was then interpreted to determine an estimate of the impact of CDAD in the United States. The results stated that a total of 15,461 cases of CDAD were found in the ten regions studied with 65.8% of the infections being health-care associated. Ironically, only 24.2% of those had onset during hospitalization. The demographic data stated that of those affected, there was a higher ratio of females to males with CDAD. Caucasians were more commonly affected than other races, and those 65 or older had a higher prevalence of CDAD (Lessa et al. 2015).
The estimated number of deaths in 2015 was calculated to be 29,300 nationwide (Lessa et al. 2015). The mortality estimation indicates the precedence this disease should take in doctors and the communities lives.

Symptoms of Disease

The signs for mild cases of CDAD include watery diarrhea occurring three or more times a day for at least two days and occasional stomach cramping with tenderness. A severe infection would cause the symptoms of watery diarrhea to occur 10 to 15 times a day, more frequent stomach cramps with some perceived as sharp, fever, blood or pus in stool, nausea, and dehydration (Heinlen & Ballard, 2011).

Diarrhea occurring at this frequency will cause dehydration rapidly. This symptom needs special attention as severe dehydration can lead to hospitalization to replenish fluids via IV. The fever is caused by the immune response to the presence of the bacteria. Interleukin-6 is activated to increase body temperature to inflict stress on the invaders causing them to replicate at a slower rate or to halt replication all together (Evans, Repasky, and Fisher, 2015). Blood in the stool is a sign of pseudomembranous colitis as cells are being destroyed in the colon, creating ulcers. The innate response to bacterial infection causes pus as an attempt to trap the bacteria and all macrophages and other immune responses to attack it (Pisetsky, 2011). Once the toxins have destroyed the initial layers of epithelial cells, they will reach blood vessels causing blood loss as the toxins destroy these cells as well. This complication can also cause concern for excessive blood loss. If the colitis is not treated the person affected may need a blood transfusion to replace the blood that was lost.

Role of Normal Microflora

There are over 100 trillion microbes that live in the gut and help the body fight against diseases and aid in food digestion (Amon & Sanderson, 2017). The human body provides nutrients and a stable environment for the bacteria to survive and thrive. The intestines provide an anaerobic environment which is suitable for the bacteria. This collection of bacteria present in the colon provides humans the ability to digest foods that generally would be undigestible, while

also aiding in the absorption of nutrients. There are also bacteria present that do not assist in the breakdown of substances and instead release vitamins beneficial to the human body. Studies have been done that have indicated the presence of the bacterium that can also sense cells that are pre-carcinogenic or capable of producing a mutation and acting on them. Research is continuously being done to determine the exact composition of the microbiome and what each specific bacteria's function is concerning the human body.

Broad Spectrum Antibiotics in Disease

Broad-spectrum antibiotics are commonly used today to treat common illnesses like skin infections, respiratory infections, and other less severe infections. They are beneficial to use when a doctor may not know what specific bacterium has invaded the host or many bacteria could cause the infection. Instead of running lab tests, which cost money and take time, the doctor can prescribe broad-spectrum antibiotics that target a particular subset of bacteria that are generally responsible for causing certain infections. Many factors need to be taken into consideration when prescribing antibiotic therapy.

It is essential to monitor the levels of antibiotic-resistance present continually or emerging in *C. difficile* isolates. Many bacteria can adapt and change their genome to prevent an antibiotic from eradicating them. This situation frequently occurs, with many bacteria including *C. difficile*. Medications like clindamycin and cephalosporins are commonly used to treat other bacterial infections, but due to resistance emerging in this organism, are no longer used to treat *C. difficile* infections (Slimings & Riley, 2014). Cephalosporins and quinolines are also common antibiotics that fight bacterial infections, but have had a correlation with causing overgrowth of *C.* difficile (Deshpande A *et al.* 2013). While these antibiotics are clearing the target bacterial infection, they are potentially promoting the colonization of *C. difficile*. People taking these

medications are more likely to be affected by CDAD than with other antibiotics (Wilcox et al. 2016). The emergence of the virulent NAP-1/027 strain has been associated with fluoroquinolone resistance as well (Kelly and Lamont, 2008).

Treatments

It has become difficult to decide how to treat a bacterial infection without running the risk of causing a CDAD. Also, there are only a limited number of antibiotics that do not effectively promote CDAD, yet *C. difficile* is becoming resistant to these antibiotics. Furthermore, antibiotics only inhibit vegetative bacteria and do not affect endospores. Given the extenuating circumstances and rate of occurrence, alternative treatments are being researched and evaluated to find a cure for CDAD.

Prevention is one of the best methods to reduce the rate of colonization and infection by *C. difficile*. Besides proper cleaning techniques in hospitals or long-term care facilities, adequate handwashing is a useful tool to stop the spread of *C. difficile*. One should always make sure to wash their hands each time they use the restroom. Hospital staff and visitors should wash their hands before and after dealing with a patient. Even if they did not physically touch the patient, they could have still come into contact with the bacterium. Proper hand washing technique includes getting one's hands wet with clean running water and using soap. One should rub their hands together to create a lather of soap for approximately 20 seconds. Ensure everywhere on the hand has been washed with soap, even under the fingernails. Then proceed to rinse with clean running water and dry hands using an air dryer or clean paper towels (Mathur, 2011).

Probiotics

The microbiome is extremely important for human health. The microbiome or microflora is affected as a side effect of long-term antibiotic use. The antibiotic may get rid of the disease-

causing bacteria, but it also gets rid of normal protective microbial flora. In this state, the body cannot defend against *C. difficile* present in the colon. One of the ways people are looking into treating CDAD is with the use of probiotics to supply the gastrointestinal tract with good bacteria. Probiotics are supposed to increase the number of bacteria present, leading to a stronger microbiome. A small number of studies indicate that strains of bacteria including *Lactobacillus acidophilus* and *Lactobacillus casei* have been effective in reducing the rate of progression of antibiotic-associated diarrhea and Saccharomyces boulardii treatment alone was helpful in the treatment of CDAD (McFarland, 1995) (Gao *et al.*, 2010). Another study used the probiotic Lactobacillus plantarum on patients who had developed CDAD during extended hospitalization and was administered at the same time that the patients were to start antibiotic therapy. This probiotic helped reduce the incidence Of CDAD while the patients remained on long-term antibiotics to treat other complications (Dudzicz et al. 2018). Some of these studies seem hopeful, but many of them are small scale studies. They lack the numbers needed to prove that CDAD can be prevented or treated with probiotics to a diverse population. There are also many studies conducted that show that the use of probiotics did not address CDAD. The amount of studies conducted in this area of health seems minute in comparison to other diseases and treatment methods and research needs to be continued to determine the effectiveness of probiotics.

Antibiotics

Even though infections are caused by long term antibiotic therapy, current treatments for CDAD require antibiotics. In some cases, CDAD could be treated by stopping current antibiotic therapy to allow the return of the normal microbial flora. Many people cannot stop antibiotic therapy because they need this antibiotic to fight another disease attacking their body. Many

people with severe infections like bone or heart infections cannot stop taking the antibiotic because it would cause the other infection they are also fighting to thrive and become lethal. Antibiotics like metronidazole and vancomycin have been used as temporary fixes for the treatment of infections caused by *C. difficile*. Metronidazole is currently used for the milder cases of CDAD and vancomycin is used to treat more severe cases. (Bakken *et al.* 2011). Many scientists and doctors worry that this treatment will only create a strain of bacterium that will also be resistant to the only effective antibiotics. Once this occurs, we will no longer have a single effective medication to treat CDAD.

Fecal Transplant

A relatively new way to treat reoccurring CDAD is to perform fecal transplantation. Fecal implantation is not performed until after the two antibiotics mentioned above have been used and failed at either getting rid of CDAD or the disease came back shortly after finishing antibiotic therapy. Reoccurrence occurs in 30% of individuals treated for *C. difficile*. Vancomycin can also have long term side effects like altering the composition of the microbial flora, which some studies found to be more severe compared to Metronidazole (Lewis *et al.* 2015). Others stating that fecal microbiota therapy had better results in treating reoccurring CDAD when compared to treatment with traditional antibiotics like vancomycin (Brandt, 2012). The monetary aspect of these two treatments was also evaluated to determine if one was less expensive than the other. It is essential to include the quality of life when making choices based on monetary cost as well. Fecal implantation was also less costly and more effective at treating recurrent CDAD when compared to vancomycin treatment. The opportunity for recurrent CDAD after treatment with vancomycin was included when calculating the overall cost (Health Quality Ontario, 2016).

Those needing a transplant will need to work along with their doctor to find a donor that matches with their specific health requirements like blood type. The transplant occurs using a colonoscopy procedure. The camera scope will carry and release the sample into the colon once the appropriate region is reached. (Bakken *et al.* 2011) This treatment has shown to be approximately 90% effective in ridding of recurrent CDAD (Brandt, 2012). More research will need to be done on this method before it is implemented more routinely.

Conclusion

CDAD is becoming a more common, and this appears to be driven by alterations in the causative agent bacterium that latter's its virulence factors and susceptibility to antibiotics. Many people are projected to become infected with strains that express increased virulence. Without a proper way to treat the disease, mortality rates will continue to climb. Most of the antibiotics once implemented to address this disease are no longer useful, and there are only a few antibiotics left to treat CDAD. Alternative treatment methods are needed to overcome the resistance hurdle, but more research is required before any approach can be adopted into a standard treatment. More research is necessary in order to develop novel antimicrobial therapies, or new antibiotics, to treat this disease, and clearly, infection control measures in hospitals need to be maintained. Fecal implantation has also been shown to appear very effective in treating CDAD, but much more research is required to confirm these findings

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