ABSTRACT

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**Alternative Strategies in the Treatment of Clostridioides difficile Infection**

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**Author’s contribution**

The sole author designed, analyzed, interpreted and prepared the manuscript.

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**ABSTRACT**

**Clostridioides difficile** (C. diff.) is a leading cause of nosocomial infections worldwide and is a major challenge to public health. Widespread use of antibiotic agents have caused increasing incidence rates of C. diff. infections and the emergence of antibiotic-resistant strains of the potentially-deadly bacteria. The current treatment guidelines include the use of various antibiotics, which further contributes to the problem of antibiotic resistance. There is an urgent need for novel treatment methods in order to halt the emergence of even more antibiotic-resistant bacteria. This review discusses the pathogenesis of C. diff. infections, current treatment strategies, and possible alternative treatment strategies based on breakthrough scientific research.

**Keywords:** Clostridioides difficile; infection; antibiotic resistance; alternative treatment; nosocomial infection.

**1. INTRODUCTION**

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conditions, including resistance to surface cleaning and standard ethanol-based sanitizers. This allows the spores to survive in hospital and healthcare settings for long periods of time, making them highly transmissible and infectious [3]. C. diff. infection occurs mainly through the fecal-oral route, often from exposure to hospital equipment or surfaces contaminated with C. diff. spores [4]. The dormant spores make their way through the gastrointestinal tract and actively grow to their vegetative form when exposed to different constituents of bile [5]. The vegetative form of C. diff. produces and releases toxins into the colonic environment that causes diarrhea, also known as Clostridioides difficile-toxins (CDT). Standard treatment protocols mainly call for the use of certain antibiotic agents, but the use of these antibiotic agents are also risk factors for C. diff. infections. Overuse of these antibiotic agents also contribute to the growing problem of antibiotic-resistant strains of many types of bacteria including C. diff. itself, making certain bacterial infections increasingly difficult to treat. It is therefore important to find alternative treatment strategies that do not contribute to antibiotic resistance.

2. PATHOGENESIS OF CDAD

Although C. diff. toxins can cause severe diarrhea, the bacteria itself is present in the microbiome of some healthy individuals without causing symptoms. Research has shown that C. diff. can asymptomatically colonize the gut of 0-17.5% of healthy adults [6]. This implies that there are many other factors involved in the development of CDAD, not just the presence of C. diff. in the gut. This section will discuss the various factors involved in the pathogenesis of CDAD.

2.1 Colonization and Infection

C. diff. colonizes the gut by the transmission of its spores starting at the mouth through to the large intestine. The spores are resistant to acids in the stomach, but start to germinate once they come into contact with different bile constituents further down the gastrointestinal tract. Fortunately, for healthy adults with an intact gut microbiome that has not been treated with antibiotic agents, C. diff. has difficulty establishing itself in the large intestine. Although C. diff. may still be present in the large intestine, it is kept under control by the complex interactions between the bacteria in the microbiome, and between the microbiome and the body. Research studies have shown that antibiotic treatments can induce changes in the microbiome and metabolome that increase susceptibility to C. diff. infection [7]. These changes include an increase in the abundance of taurocholate, a bile acid used by C. diff. for germination, and also an increase in carbon sources like stachyose, raffinose, sorbitol, fructose, and mannitol which are all used by C. diff. for growth [7]. All of these changes lead to an environment favorable to the colonization of C. diff. and subsequent infection [7]. CDAD occurs when the vegetative form of C. diff. produces toxins in the large intestine that cause changes to the intestinal epithelial cells leading to symptoms like diarrhea, abdominal pain, and fever.

2.2 Toxin-Mediated Damage

Toxin A (TcdA), Toxin B (TcdB), and C. difficile transferase toxin (CDT) are three of the toxins produced by C. difficile [8-9]. TcdA and TcdB are the two toxins that are highly researched, due to their responsibility for causing the cellular changes leading to the symptoms of CDAD [8]. On the other hand, it is still unclear what role CDT plays on CDAD progression, although some research studies have shown that the toxin increases bacterial adherence on intestinal epithelial cells, implicating a role in C. diff. colonization. Not all strains of C. diff. produce CDT, but many of the strains isolated from patients with severe CDAD are able to produce this toxin [9].

The two main virulence factors in C. diff. are TcdA and TcdB, glucosyltransferases that enter colonic epithelial cells by receptor-mediated endocytosis before deactivating Rho-family GTPases like Rac, and Cdc42 [8]. Inactivation of these cellular proteins result in cell apoptosis due to structural disruption. Recent research has shown that TcdA and TcdB often work together to induce cell damage and death, but the absence of either toxin can still result in disease. A recent study observed the activities of both toxins side by side and found that TcdA causes apoptosis in target cells dependent on the glucosyltransferase activity of the toxin, while TcdB works differently at low and high concentrations [10]. At low concentrations, TcdB is dependent on glucosyltransferase activity to induce cell apoptosis, while at higher concentrations, it works independently of its glucosyltransferase activity to cause cell death by producing reactive oxygen species [10]. The
The presence of either TcdA or TcdB alone in the gut is enough to produce the full spectrum of symptoms associated with CDAD [11].

TcdA and TcdB can act on many different mechanisms to cause disease. In addition to inducing necrosis in intestinal epithelial cells, TcdA and TcdB also degrades and inactivates YAP and TAZ proteins in the cytoplasm. YAP and TAZ are two proteins which act as transcriptional coactivators downstream of the Hippo pathway, which is responsible for tissue homeostasis and intestinal regeneration [12]. TcdB has also been shown to damage stem cells in the colon, inhibiting the ability of intestinal epithelial cells to repair itself [13]. By impairing the colon’s ability to repair and regenerate on a cellular level, C. diff. toxins create an environment suitable for chronic disease.

### 2.3 Disease Presentation

The presence of C. diff. in the gut can result in a variety of clinical presentations, ranging from being an asymptomatic carrier to having severe, potentially fatal fulminant colitis or toxic megacolon. Infection by C. diff. can occur after starting antibiotic therapy, often presenting as mild to moderate diarrhea without blood. Abdominal cramps and anorexia may also accompany the diarrhea [14]. In severe cases, colonoscopy will reveal pseudomembranes in the large intestine, presenting as elevated colonic mucosal nodules or plaques that are white-yellow in color [15]. Severe cases will also present with fever, dehydration, electrolyte imbalance, leukocytosis, and hypoalbuminemia. In cases where CDAD has progressed to toxic megacolon or fulminant colitis, there will be tenderness and distention in the abdominal area, often of a severe nature [14]. Case studies have also revealed that CDAD may also have extracolonic manifestations such as small intestinal involvement, reactive arthritis, cellulitis, bacteremia, sepsis, abscesses, and more [16].

### 3. STANDARD TREATMENT STRATEGIES

The treatment strategy upon initial diagnosis of C. diff. infection is to always discontinue the antibiotic agents that may have induced the infection. Strangely enough, the standard treatment for initial CDAD is to use other antibiotic agents like metronidazole and vancomycin, with a preference for vancomycin due to its ability to reduce fecal C. diff. counts to undetectable levels. Other possible antimicrobial agents suitable for treating CDAD include fidaxomicin, rifaximin, ramoplanin, tigecycline, and nitazoxanide [17]. Unfortunately, treatment with any type of antibiotic agent, whether it be broad-spectrum or narrow-spectrum, may potentially lead to the development of antibiotic resistance.

Other uncommon treatment strategies are available to treat more severe presentations of CDAD, but are not often used due to their lack of testing or high price point. Some of these treatment strategies include administration of immunoglobulins, monoclonal antibodies, or vaccines [17]. Immunoglobulins can be administered orally or intravenously, but there are very few research studies with this treatment strategy [18]. Studies have shown promising results from treatment of CDAD with immunoglobulins, but intravenous immunoglobulin therapy is prohibitively expensive. Oral administration of immunoglobulins is promising but needs further studies to show clinical efficacy [18].

Administration of monoclonal antibodies is another possible treatment, but is not commonly used for CDAD. Bezlotoxumab is a monoclonal antibody that specifically targets antigens present on the surface of TcdB, resulting in a very effective treatment to prevent recurrent C. diff. infection. As of 2017, Bezlotoxumab is the only United States Food and Drug Administration approved monoclonal antibody for C. diff. infection [19]. It is usually administered intravenously as an adjunct treatment to metronidazole. Unfortunately, Bezlotoxumab is extremely expensive at $3800 per vial, in addition to the other costs associated with administration of the treatment [20].

There are a number of vaccines for the prevention of CDAD in development, but none have been approved for official use yet. Similar to monoclonal antibodies, vaccines for CDAD target the toxins produced by C. diff., not the bacteria itself. Vaccines for CDAD use recombinant or detoxified forms of TcdA or TcdB to instruct the body to create antibodies to help neutralize the toxins. Unfortunately, this approach alone only decreases the symptoms associated with CDAD, but does not inhibit C. diff.’s ability to germinate, colonize, and infect the gut. Because of this, CDAD vaccines have to be used in conjunction with other protocols like antibiotic treatment [21].

Currently, the most promising treatment for CDAD is fecal microbiota transplant (FMT),
having shown good results in many clinical studies. FMT involves the transplant of fecal material from a healthy donor into the colon of diseased individuals. This process reintroduces beneficial gut bacteria into the colon of patients with CDAD, increasing bacterial diversity and essentially renewing their gut microbiome to be able to more effectively control the population of *C. diff.* and toxin production [22]. Several routes of administration are available, including enema, colonoscopy, nasogastric tube, and capsules. A recent study compared the efficacy of all four methods of FMT and found that administration by colonoscopy was superior to nasogastric tube and enema, while administration by capsules was comparable to colonoscopy [23]. Unfortunately, the *C. diff.* infection clinical practice guideline released by the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America recommends antibiotic therapy as the standard protocol for CDAD and only recommends FMT after multiple failed rounds of antibiotic therapy, indicating recurrent infection [24]. This treatment recommendation inadvertently contributes to the growing problem of the development of antibiotic-resistant strains of *C. diff.*

4. POSSIBLE ALTERNATIVE TREATMENT STRATEGIES

Alternative medical systems like Traditional Chinese Medicine (TCM) have been used to successfully treat disorders like diarrhea for millennia. There are many TCM herbal formulas that can treat various presentations of diarrhea. It comes as no surprise that some of the herbal components within these formulas have antimicrobial properties against *C. diff.* and other diarrhea-causing microbes. Plant-based remedies often target bacteria through mechanisms different than conventional antibiotics and can act as resistance modifying agents, which are compounds that act against bacterial resistance [25]. Not only would these plant-based remedies help resolve the issue of antibiotic resistance, they would also help prevent the development of more difficult-to-control resistant strains.

Similar to strategies of orthodox medicine, there are many different approaches to target *C. diff.* with natural remedies in the treatment of CDAD. Some of these approaches include using herbs that have been shown to have bactericidal or bacteriostatic activity against *C. diff.*, using herbs that can neutralize *C. diff.* spores, using herbs that can counteract TcdA and TcdB activity, and others. Below are some of the alternative remedies that have been shown in laboratory settings to have potential therapeutic value in treating CDAD.

4.1 Shen Ling Bai Zhu San (Ginseng, Poria, and Atractylodes Macrocephela Powder)

Shen Ling Bai Zhu San (SLBZS) is a very commonly used TCM herbal formula for the treatment of various gastrointestinal issues, such as diarrhea and poor appetite. This formula dates back to the Song dynasty, where it was first documented in the “Tai Pin Hui Min He Ji Ju Fang”, which translates to “Formulary of the Pharmacy Service for Benefiting the People in the Taiping Era” [26]. SLBZS has the following ten herbs: Ren Shen (Radix Ginseng), Bai Zhu (Rhizoma Atractylodis Macrocephalae), Fu Ling (Poria Cocos), Zhi Gan Cao (Radix Glycyrrhizae Preparata), Shan Yao (Radix Dioscoreae), Bai Bian Dou (Semen Dolichorhous), Lian Zi (Semem Nelumbinis), Yi Yi Ren (Semen Coicis), Sha Ren (Fructus Amomi), and Jie Geng (Radix Platycodi). In vitro studies have shown that SLBZS has bacteriostatic activities against different strains of *C. diff.*, inhibiting its growth at various concentrations [27]. Research has shown that the microbiome diversity is decreased in the gut of patients with CDAD [28]. Expectedly, SLBZS has also been shown to alter the gut microbiome, increasing its diversity and restoring its balance [29]. These actions show a lot of promise, but SLBZS needs further in-vivo studies to confirm its efficacy in the treatment of CDAD.

4.2 Qi Pi Yi Fei (Arousing the Spleen and Tonifying the Lung)

Qi Pi Yi Fei (QPYF) is a TCM herbal formula used for treating antibiotic-associated diarrhea. QPYF has the following seven herbs: Hong Jing Tian (Rhodiola Rosea), Fu Ling (Poria Cocos), Dang Shen (Codonopsis Pilosula), Bai Zhu (Atractylodes Macrocephala), Ge Gen (Radix Puerariae), Sheng Jiang (Rhizoma Zingiberis), and Gan Cao (Radix Glycyrrhizae). This formula was studied on a *C. diff.*-associated diarrhea mouse model, where mice were given a mixture of different antibiotic agents including kanamycin, gentamicin, colistin, metronidazole, vancomycin, and clindamycin before being infected with *C. diff.* by oral gavage [30]. In this research, an experimental group of mice were given QPYF...
seven days prior to C. diff. infection as a preventative measure. Although this study did not directly evaluate the effects of QPYF on C. diff., it showed promising results in the prevention of CDAD caused by antibiotic therapy. Histopathological analysis showed reduced colon tissue damage and immunohistochemical analysis showed reduced expression of the disease pathways tumor necrosis factor-alpha, monocyte chemoattractant protein-1, nuclear factor kappa-light-chain-enhancer of activated B cells p65, and phosphorylated-nuclear factor-kappa-light-chain-enhancer of activated B cells p65. Clinical symptoms like diarrhea and weight loss were improved and mortality was reduced. Toxin secretion was also reduced as shown by toxin analysis. Overall, this study shows that QPYF given preventatively may help treat CDAD in mice [30]. Clinical trials with this formula may further determine its clinical efficacy in humans.

4.3 Baicalin

Baicalin is an active component found in several TCM herbs, but it is most widely associated with Huang Qin (Radix Scutellaria baicalensis). Huang Qin itself is an herb that can treat diarrhea or dysentery and is also included in many herbal formulas that treat various gastrointestinal issues. A recent in-vitro study explored the efficacy of baicalin’s ability to reduce C. diff. sporulation and toxin production and found that its subinhibitory concentration significantly reduced C. diff. spore outgrowth, sporulation, and toxin synthesis. The study also found that baicalin significantly downregulated gene expression in C. diff. critical for its pathogenesis [31]. Further in-vivo studies and clinical trials may confirm if baicalin has the ability to help control CDAD in humans.

4.4 Berberine

Berberine is an active component found in several TCM herbs, but it is most widely associated with Huang Lian (Rhizoma Coptidis). Huang Lian itself is an herb that can treat diarrhea or dysentery with or without bleeding. Like Huang Qin, Huang Lian is also included in many herbal formulas that treat various gastrointestinal issues. A commonly administered form of berberine is berberine chloride, often found in pills or liquid form. A study found that berberine chloride had antibacterial activity against all of the C. diff. strains investigated but did not affect their spores. Although the spores could not be killed, berberine chloride was able to block the outgrowth of spores that started to germinate [32]. Further in-vivo studies and clinical trials may confirm if berberine chloride has the ability to help control CDAD in humans.

4.5 Curcuminoids

Curcuminoids are active components found in turmeric, a yellow rhizome used as a spice and food coloring in many Asian countries. It is also an herb in traditional Indian medicine and traditional Chinese medicine systems [33]. A study researching curcumin, demethoxycurcumin, and bisdemethoxycurcumin, three curcuminoids in turmeric, found that they all inhibited the growth of various C. diff. strains but did not affect spore formation. In addition, these curcuminoids inhibited toxin production and did not have negative effects on major bacteria populating the human gut like Lactobacillus, Bifidobacterium, and Bacteroides [34]. Further in-vivo studies and clinical trials may confirm if curcuminoids has the ability to help control CDAD in humans.

4.6 Manuka Honey

Manuka honey is derived from the flowers of the Manuka tree (Leptospermum scoparium) in New Zealand. It has recently attracted the attention of the medical field for its strong antimicrobial properties, due to its methylglyoxal content [35]. Unsurprisingly, Manuka honey has also been studied for its antibiotic activity against C. diff. A study found that Manuka honey has inhibitory and bactericidal activity against various C. diff. strains [36]. It can also inhibit spore proliferation but does not completely eradicate them [36]. Further in-vivo studies and clinical trials may confirm if Manuka honey has the ability to help control CDAD in humans.

5. CONCLUSION

Clostridioides difficile is a very problematic bacterium that can spread virulently and cause severe diarrhea in humans. Their spores are highly resistant to extreme conditions, allowing them to survive and thrive when the right conditions become available. C. diff.’s treatment with antibiotics like metronidazole and vancomycin have resulted in the development of antibiotic resistant strains, further adding to its virulence and difficulty in treatment. Immunoglobulins, monoclonal antibodies, and
vaccines are all treatments and methods of prevention that may avoid the development of bacterial resistance, but are either restricted by their high price point or their need to be combined with antibiotic therapy, again adding to the risk of creating additional antibiotic resistant strains. Fecal microbiota transplant is currently one of the most promising treatment strategies for CDAD, but is often preceded by the administration of multiple rounds of antibiotic agents. Alternative medical systems like Traditional Chinese Medicine have been treating the symptoms associated with CDAD for millennia, but have not yet been thoroughly studied in the framework of orthodox biomedical sciences. Although this is the case, researchers have already begun to realize the promising potential in these alternative treatment strategies for CDAD. Herbal formulas like Shen Ling Bai Zhu San and Qi Pi Yi Fei have already been shown to have inhibitory effects on C. diff. or toxin reduction. Herbal components like baicalin, berberine, and curcuminoids have been shown to possess sporicidal or bactericidal properties against C. diff. with an ability to inhibit toxin-mediated damage. Natural medicine products like Manuka honey have been shown to have bactericidal activity against C. diff. and could help inhibit their spore proliferation. There are still many herbs, formulas, and natural medicine products that could potentially treat CDAD waiting to be studied. These may be part of the next wave of promising treatment strategies that may finally end the problem to severe C. diff. infection and antibiotic resistance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES


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