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Review Article

A Review on the Effects of Probiotics and Antibiotics towards *Clostridium difficile* Infections

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ABSTRACT

Clostridium difficile can cause severe diseases with significant morbidity and mortality in infected patients. The rate of *Clostridium difficile* infection is high in North America and European countries. Metronidazole and vancomycin have been recommended as the treatments of choice since 1990s. Recurrent infection due to *Clostridium difficile* is common after several days of antibiotic administration. Probiotics have been used in these patients as an adjunct treatment with some successful findings. However, a detailed investigation on the use of probiotic for infected patients is still needed, particularly for its real efficacy.

Keywords: Clostridium difficile, Probiotic, Antibiotic

INTRODUCTION

Clostridium difficile is one of the colonic microfloras which can be categorised as opportunistic pathogen, obligate anaerobe, Gram-positive and spore-forming bacterium (Bruno *et al*., 2006; Pituch, 2009). Approximately 3% of *C. difficile* tends to be found in intestines of healthy adults and 40% in neonates (Libby *et al*., 2009).

In 1978, *C. difficile* was identified as one of the opportunistic pathogens to have caused diarrhoea (Huang *et al*., 2009). It was reported that this bacterium responsible for 10-25% antibiotic-associated diarrhoea, 50-75% antibiotic-associated colitis and 90-100% cases of antibiotic-associated

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pseudomembranous colitis, respectively (McMaster-Baxter *et al.,* 2007).

C. difficile produces two main toxins, namely, enterotoxin A (TcdA) and cytotoxin B (TcdB) (Pituch, 2009). These toxins are among the virulent factors that are responsible for the pathogenesis of antibiotic-associated diarrhoea and pseudomembranous colitis (Pituch, 2009).

The bacteria can be further classified as non-toxigenic strains [A^-B^-] (not producing both toxins), toxigenic strains [A^+B^+] (i.e. producing both toxins), and [A^-B^+] strains (producing only toxin TcdB) (Rupnik *et al*., 2003). In addition, *C. difficile* also produces toxin called binary toxin CDT. This toxin is composed of two non-linked components: CDTa (enzymatic component) and CDTb (binding component) (Rupnik *et al*., 2003). However, the pathogenicity for this toxin is still unknown (Rupnik *et al*., 2003).

EPIDEMIOLOGY

Prior to 2001, epidemiological information was limited due to mild manifestation and inexpensive treatment of *C. difficile* infection [CDI] (Oughton *et al*., 2008). After 2001, however, the incidences of *Clostridium difficile*-associated diarrhoea [CDAD] had increased drastically to 26% in U.S. hospitals (Miller *et al*., 2006). A study by Walbrown *et al*. (2008) in North America showed more than 250,000 cases of CDAD in European countries, and the experience of CDI was almost similar in North America (Oughton *et al*., 2008; Denève *et al*., 2009). The information about *C. difficile* and CDAD was also limited in Asian countries (Oughton *et al*., 2008). For instance in Japan, the epidemiology of CDAD has not been well documented (Sawabe *et al*., 2007). In Malaysia, data on CDI is rather limited. The only documented data that could be traced was published by Parasakthi *et al*. (1988) who reported seven cases of CDAD.

CDAD does not only affect adults but it can also have few impacts on infants and neonates. It has been reported that 26.6 - 43.5% of neonates and infants in Europe and USA are carriers of *C. difficile* (Pituch, 2009). In Japan, the carrier rates for *C. difficile* were 84.4% and 30.3% in infants under 2 years old were 84.4% and 30.3% for children more than 2 year old (Pituch, 2009).

There are several *C. difficile* strains that can cause CDI. For instance, *C. difficile* strain 027 causes severe colitis, higher mortality and higher recurrent rates (CA Clements *et al*., 2010; Dawson *et al*., 2009). This strain can produce higher levels of TcdA and TcdB (Denève *et al*., 2009). In UK, strain 027 has increased from 25.9% to 41.3% and the increase pattern was similar to that in Canada where it reached 75.2% by the year 2003. In Japan, strain 027 infection was documented in a hospitalized patient in 2005 (Oughton *et al*., 2008). This strain was first detected in several cases reported from in Western Australia, South Korea, Hong Kong and Costa Rica were reported between 2008 and 2010 (Clements *et al*., 2010). Moreover in Japan, the most common strain that was responsible for causing outbreaks in several hospitals was PCR ribotype smz (Sawabe *et al*., 2007). This PCR ribotype smz has been identified from the analysis of 148 isolates in a Japanese teaching hospital (Oughton *et al*., 2008).

IMMUNE RESPONSE TO CDI

Patients with *C. difficile* diarrhoea have decreased amount of serum IgG antitoxin A (Wilcox, 2003). This antibody is important to reduce the severity of symptoms and reduce the rate of recurrent diarrhoea (McMaster-Baxter *et al*., 2007).

Several studies have examined the success developmental rate of immune response towards *C. difficile* toxins in recurrent CDAD (RCDAD) patients. Huebner *et al*. (2006) demonstrated that when intravenous immunoglobulin (IVIG) was given to five children who had RCDAD, their IgG antitoxin A levels were increased. Meanwhile, when IVIG was administered to five adult RCDAD patients, three of them had no further recurrence with one had a recurrence while one had died of uncontrolled CDAD (Huebner *et al*., 2006).

Orally administered antibodies have been used for humans but their effectiveness is still inconclusive. Whey protein concentrate from the milk of *C. difficile*-immunised cows contains high level of IgA antibodies. It is safe and is able to decrease the recurrence rates up to 50% (Bauer *et al*., 2009a). However in another study, nine out of 16 CDAD patients developed RCDAD when it was given to them (Huebner *et al*., 2006).

Immunoglobulins from the colostrums of cows immunised with *C. difficle* toxoids were used to neutralize the effects of toxins A and B *in-vitro* and had been shown to inhibit the enterotoxic effects in a hamster CDI model (Huebner *et al*., 2006; Bauer *et al*., 2009a). In addition, anti-toxin A antibodies and anti-toxin B antibodies were also found to inhibit the effects of CDAD although the mechanism of immune response to toxin B has not been well understood (Mylonakis *et al*., 2001).

Clostridium difficile ASSOCIATED DIARRHOEA (CDAD) AND PSEUDOMEMBRANOUS COLITIS

CDAD and pseudomembranous colitis are among the common diseases caused by CDI in hospitalized patients. The incidence and severity of CDAD have been shown to be increased in many studies. In the United States, for instance, this disease was identified as a major cause for hospital-acquired diarrhoea, especially among patients in long-term care facilities. The disease was also seen among patients aged 65 years and above (McCusker *et al*., 2003; Mylonakis *et al*., 2001; Pelleschi, 2008).

Usually, the colonic microflora of healthy adults is resistant to *C. difficile* colonization (Poutanen *et al*., 2004). However, the protection conferred by the colonic microflora will decrease when patients are treated with antibiotics. This causes an increase in the number of *C. difficile* in the colon, which may lead to severe diarrhoea or pseudomembranous colitis (McMaster-Baxter et al., 2007; Pelleschi, 2008).

The aetiology for CDAD is believed due to the release of to be toxins A and B (Pelleschi, 2008). When the spores enter the colon, *C. difficile* will proliferate and release toxins A and B (Miller *et al*., 2006). Once the toxins are secreted, toxin A will activate macrophages and mast cells and subsequently the inflammatory mediators will be released. Activation of these cells causes disruption of the cell wall junction, resulting in an increase in the permeability of the intestinal wall and subsequently diarrhoea. Meanwhile, toxin B degrades the epithelial cells in colon (Pelleschi, 2008). In addition, production of hydrolytic enzymes

leads to pseudomembranous colitis and watery bloody diarrhoea (Miller *et al*., 2006). Pseudomembranous colitis is a term used for severe CDI (Wilcox, 2003). Most patients will present with severe diarrhoea. It usually occurs within 1-2 weeks after the commencement of broad-spectrum antibiotics (Huebner *et al*., 2006).

SIGNS AND SYMPTOMS OF CDAD AND PSEUDOMEMBRANOUS COLITIS

The clinical manifestations of CDAD range from mild to severe symptoms which include malaise, lower abdominal cramps, nausea, vomiting, fever, loss of appetite and leukocytosis (Miller *et al*., 2006; Koo, 2008; Mylonakis *et al*., 2001). The symptoms of CDAD may appear on the first day or up to 6 weeks of the antibiotic therapy (Mylonakis *et al*., 2001). The stool can become watery, voluminous and non-bloody (McMaster-Baxter *et al*., 2007). As for pseudomembranous colitis, the common signs and symptoms are painful abdominal distension, watery diarrhoea, fever, and colonic bleeding (Tsourous *et al*., 2007; Mylonakis *et al*., 2001).

DIAGNOSIS

Currently, CDI can be diagnosed either by laboratory methods or endoscopy (Wilcox, 2003). In laboratory diagnosis, the bacterium is isolated and cultured but this method is very time consuming. However, epidemiology of *C.difficile* strains in a ward or hospital is determined by using this method.

The detection of the toxin B cytotoxicity can be performed by using tissue culture assay (Mylonakis *et al*., 2001). This test is the most sensitive (94%-100%) and specific (99%) for diagnosing CDI but it takes approximately 1 to 3 days (Mylonakis *et al*., 2001). The most frequently used method to detect the toxins of *C. difficile* is by ELISA test kit, which is easy to perform but it has low level of sensitivity (Wilcox, 2003; Mylonakis *et al*., 2001). Immunoassays are known to be the best method to perform cell cytotoxicity assay with sensitivity and specificity in the range of 85-95%. Toxins tcdA, tcdB, cdtA and cdtB can also be detected by using real-time polymerase chain reaction (RT-PCR) (Huang *et al*., 2009).

Diagnosis by sigmoidoscopy remains as the optimum way of distinguishing different pathological conditions in the large intestine. Common sigmoidoscopic findings are yellow adherent plaques measuring 2-10 mm in diameter, which are formed by pseudomembranes that are found scattered over the colonic mucosa and interspersed with hyperemic mucosa (Bonasera *et al*., 2004).

ANTIBIOTIC SUSCEPTIBILITY

Currently, antimicrobial therapy is the mainstay of treatment for CDI (Huang *et al*., 2009). Many studies have shown the use of metronidazole and vancomycin as the primary treatment for CDI as they are the most active agents *in vitro*, with narrow MIC_{50} and MIC_{90} ranges (Huang *et al*., 2008). Metronidazole commonly acts as the first line treatment, while vancomycin is normally used for the second line therapy (Johnson, 2009).

Fluoroquinolones and rifaximin have also been used in CDI treatment (Johnson *et al*., 2007; McCusker *et al*., 2003). Cases of fluoroquinolones-resistant *C. difficile* have been reported

in CDI (Huang *et al*., 2009). For example, 37.5%, 46.4%, 12% and 7% of *C. difficile* isolates have been reported to be resistant to moxifloxacin in European countries, Shanghai, Germany and France, respectively (Huang *et al.*, 2009). Of interesting note, the use of fluoroquinolones has been documented as one of the main risk factors for CDAD (Miller *et al.*, 2006; McCusker *et al.*, 2003).

Rifaximin is commonly used for the treatment of patients with traveller's diarrhea. However, this antibiotic can also be used to treat CDI as well. A study had reported that eight patients who had multiple RCDAD responded to rifaximin, except for one person who needed a second administration of rifaximin for complete resolution (Johnson, 2009).

Broad-spectrum antibiotics such as penicillins, cephalosporins and clindamycin can contribute to the changes of colonic microflora and cause the continuation of CDAD (Surawicz, 2003; Poutanen *et al*., 2004). In addition, the administration of meropenem and piperacillin or tazobactam may have an effect on other gut flora and probably increase the risk of CDI (Huang *et al*., 2008).

Giving antibiotics to all CDI patients is not a routine practice. This is because some patients will recover spontaneously from the disease but this happens mainly in mild cases of CDI. Thirty three percent of mild CDI patients had spontaneous recovery (Bauer *et al.*, 2009b). Meanwhile, other studies showed that once the antibiotics were discontinued, 15-23% of CDAD patients had spontaneous recovery (McMaster-Baxter *et al.*, 2007). However, there were possibilities that the recurrence would be increased if patients took another antibiotic for the second time after discontinuing the first antibiotic (Bauer *et al.*, 2009b).

One of the problems of antibiotic usage is resistance. There were reports showing 7.7% and 6.3% cases of antibiotic resistance towards metronidazole in Spain in 1994 and 2002, respectively. There was a drastic increase from 20% to 47.2% in Canada, and this occurred after the patients had received metronidazole (Miller *et al.*, 2006).

There was also resistance to vancomycin and this has been increased every year (Huang *et al*., 2009). For example in Scotland, it has been reported that the increase of vancomycin resistance was from 2.7% (1999-2000) to 21.6% (2005) (Huang *et al.*, 2009). According to McMaster-Baxter et al. (2007), 3% of *C. difficile* isolates had intermediate level of resistance towards vancomycin; however the clinical implication of this finding was not studied.

RECURRENCES OF CDI AND PROBIOTIC

Recurrent CDI has seldom encountered in hospitalized patients. This usually occurs in 20-35% of the patients post 5-8 days of antibiotic therapy (Huebner *et al.*, 2006). It had been stated that the recurrence could probably be due to the decrease in the colonisation resistance (Johnson, 2009). Apart from the use of antibiotics, other known risk factors for CDI recurrence are as follows: low level of immunization (anti-toxin IgM and IgG), older age, female sex and renal disease (Johnson, 2009; Huebner *et al.*, 2006).

As an alternative, probiotics have been used as an adjunct treatment for CDI. Probiotics are living microorganisms which can provide benefits to the host (Miller, 2009). Commonly used probiotics are *Lactobacillus* species and *Bifidobacteria* species. They are available either in yogurt or food supplements. Probiotics are also found in the human colon. Approximately

10^2-10^6 of *Lactobacillus* species and 10^8-10^{12} of *Bifidobacterium* species form parts of the intestinal flora. However, the composition of the probiotics in the colon will decrease as the subjects get older (Salminen *et al*., 2004).

Probiotics should be taken after the administration of antibiotics. Usually, the effect of probiotics will be seen over certain time duration. According to several studies, probiotics recipients had a remarkable reduction in recurrences when these were administered to them for 21 to 38 days (Huebner *et al.*, 2006).

Clinical evidence has supported the use of probiotics for the treatment of gastrointestinal infections, inflammatory bowel diseases and cancer (Saarela *et al.*, 2000). Now, many scientists believe that the use of probiotic can prevent exacerbation of CDI. For instance, *S. boulardii* has successfully reduced CDI occurrence. On the other hand, *L. rhamnosus* GG has shown mixed results in CDAD prevention (Graul *et al.*, 2009; Lawrence *et al.*, 2005).

According to Gerding *et al*. (2008) and Miller (2009), the most effective way to prevent recurrences is by combining *S. boulardii* with a standard therapy (metronidazole or vancomycin). In their reports, 11 out of 13 patients with multiple recurrences of CDI had no further recurrences. Another study has shown that the rate of RCDAD in 60 patients decreased to 50% after combining *S. boulardii* with the standard therapy (Huebner *et al*., 2006). The high dose of vancomycin, with *S. boulardii*, had reduced the recurrence of CDI; however, this would not be effective if the low dose of standard therapy was prescribed with or without the combination with *S. boulardii* (Gerding *et al*., 2008).

The combination of *Lactobacillus plantarum* 299v with metronidazole had also been used in several studies. Unfortunately, this combination did not yield good results since recurrences occurred in four out of eleven patients, as compared to six out of nine patients who had received only metronidazole and placebo (Huebner *et al*., 2006).

Studies in Valley Lutheran Medical Centre and Mesa Lutheran Hospital have demonstrated the effectiveness of combining *Lactobacillus* species and *Bifidobacterium* species on CDAD. In particular, the results showed that CDAD in the year 1999, 2000 and 2001 had decreased to 66%. This finding indicates that the combination of probiotics with antibiotic therapy could be helpful in decreasing CDAD (Graul *et al*., 2009).

There are varying opinions against the use of probiotics as an adjunct therapy. It has been reported that probiotics are not effective in treating *C. difficile* and in fact may be harmful to human. Gerding *et al*. (2008) claimed that the current literature did not support the use of probiotics for CDI. It has been reported that *Lactobacillus* may cause bacteraemia whereas *S. boulardii* may cause fungemia in both immunocompetent and immunocompromised hosts (Gerding *et al*., 2008; Miller, 2009).

OTHER ALTERNATIVE TREATMENTS

Other novel alternative treatments for CDI have also been developed. A study on vaccine was conducted among healthy volunteers. The parenteral vaccine that inactivates toxins A and B demonstrated an increase in antibody levels. A combination of vaccine with vancomycin in three patients with multiple episodes of RCDAD showed no further recurrences (Huebner *et al*., 2006).

Faecal transplant is another innovative novel treatment. This approach has been used among patients who experienced CDI recurrence. A study was carried out among 18 patients who had suffered from recurrent *C. difficile* colitis. Their stools were treated with vancomycin hydrochloric tablet (250mg) and omeprazole capsules (20mg) for four days prior to the transplant procedure. Nasogastric tube was used to transfer the faeces obtained from healthy donors to these patients. As a result, 16 out of 18 did not experience any recurrence after the treatment (Aas *et al*., 2003).

A randomized trial using faecal transplant was done in the Netherland. In that study, vancomycin was given to patients with recurrent CDI. In order to reduce *C. difficile* load, gastrointestinal lavage was performed using Kleanprep [macrogol]. The donor faeces were then transferred to the patients through a nasoduodenal tube (Bauer *et al*., 2009a). Johnson (2009) showed that by using faecal transplant, 90% of 67 patients had reduced number of CDI recurrence.

Another alternative treatment that has been explored was tolevamer. Tolevamer is a polymer that has been tested in hamster CDI model and shown to be highly effective for C. *difficle* toxins. However, the use of this particular polymer in human needs further study (Bauer *et al*., 2009a).

CONCLUSION

C. difficle is a type of bacterium that causes a spectrum of diseases in human. The most challenging part in the CDI treatment is the recurrence cases of *C. difficile* and the high chances for patients to develop CDI recurrence. Metronidazole-resistant *C. difficile* is also need to be considered during the treatment. Thus, re-evaluating the use of metronidazole as the first-line treatment is needed in areas with high resistant rates. In addition, alternative ways should be explored to overcome this problem.

Probiotics have been shown to give some promising results in treating CDI patients. However, the use of probiotics as an adjunct treatment for CDI needs further investigations. In particular, the safety aspect and quality control of probiotics should be emphasized especially in immunocompromised patients. There is also a need for scientists to do more experiments and explore new types of probiotics. This will enable physicians to choose from a wider range of probiotics and develop a better combination to be used as adjunct treatments.

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