Dietary-based gut flora modulation against Clostridium difficile onset


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Dietary-based gut flora modulation against *Clostridium difficile* onset

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**Abstract**

*Clostridium difficile* infection is a frequent complication of antibiotic therapy in hospitalised patients, which today is attracting more attention than ever and has led to its classification as a 'superbug'. Disruption of the composition of the intestinal microflora following antibiotic treatment is an important prerequisite for overgrowth of *C. difficile* and the subsequent development of an infection. Treatment options for antibiotic-associated diarrhoea and *C. difficile*-induced colitis include administration of specific antibiotics (e.g. vancomycin), which often leads to high relapse rates. More importantly, both the rate and severity of *C. difficile*-associated diseases are increasing, with new epidemic strains of *C. difficile* often implicated. For the prevention and treatment of antibiotic-associated diarrhoea and *C. difficile* infection, several probiotic bacteria such as selected strains of lactobacilli (especially *Lactobacillus rhamnosus* GG), *Bifidobacterium longum*, and *Enterococcus faecium* and the non-pathogenic yeast *Saccharomyces boulardii* have been used. Controlled trials indicate a benefit of *S. boulardii* and *L. rhamnosus* GG as therapeutic agents when used as adjuncts to antibiotics. However, the need for more well-designed controlled trials with probiotics is explicit.

**Keywords:** probiotics, *Clostridium difficile*, diarrhoea

1. **Introduction**

*Clostridium difficile* is a ubiquitous anaerobic, sporulating, Gram-positive, rod-shaped bacterium that is isolated from both soil and living organisms, including humans (Smith and King 1962; Alpern and Dowell 1971; Hafiz et al. 1975; Hafiz and Oakley 1976; Borriello et al. 1983). One of the primary habitats for *C. difficile* is the gut of infants (Levett 1986). Prevalence rates of 46% have been reported in healthy infants under 12 months old, and colonisation rates of up to 55% have been reported in neonatal intensive care units (Donta and Myers 1982). Although in many infants faecal cytotoxic titres are extremely high and similar to those seen in adults with pseudomembranous colitis (PMC), infants show no obvious symptoms (Cooperstock and Zedd 1983). However, they are not resistant to the disease as a result of selective colonisation with non-toxigenic strains. In healthy adults, prevalence rates are rare at 3–5% (Viscidi et al. 1981), and in hospital in-patients rates are 16–35% (McFarland et al. 1989). The reasons for high colonisation rates with no symptoms in infants and low rates in adults are not clear.

2. **Importance of *C. difficile* in human infections**

In 1935, Hall and O’Toole isolated *C. difficile* from the stools of healthy newborn infants and showed strains to be toxigenic. However, the bacterium was not associated with intestinal disease until the late 1970s. Antibiotic-associated diarrhoea (AAD) is defined as diarrhoea developing from a few hours after the onset of antibiotic therapy to 6–8 weeks following antibiotic discontinuation (Bartlett 1992; Hogenauer et al. 1998). The incidence of AAD in the literature varies from 5–25% in patients receiving antibiotics, depending on the class of antibiotic used and confounding risk factors in the patients being treated (Bartlett et al. 1978a; Hove et al. 1996; McFarland 1998). After 1950 and with the development of broad spectrum antibiotics, AAD became a recognised condition. However, it was in 1978 with the emergence of PMC occurring in patients treated with clindamycin, that *C. difficile* was identified as a serious aetiological agent (Bartlett et al. 1978a, 1978b; George et al. 1978).

3. **Pathogenesis of AAD**

During most courses of antibiotic therapy, alteration of the normal flora of the bowel leads to a loss of equilibrium and consequently of the resistance to colonisation, which
may result in the emergence of pathogenic organisms such as *C. difficile* (Hogenauer et al. 1998). Disruption of the ecological equilibrium of the normal intestinal microflora may result in diarrhoea associated with alteration of fermentation processes and a reduction in short-chain fatty acids (‘functional diarrhoea’). The most important bacterial aetiology of AAD is *C. difficile* (Hove et al. 1996).

### 3.1 C. difficile and AAD

Since the demonstration of the role of this organism in PMC (Bartlett et al. 1978b; George et al. 1978), its importance has grown significantly over the last 30 years. The levels of *C. difficile* in AAD were demonstrated to be 20–25%, with over 95% of PMC involving the microorganism (Hogenauer et al. 1999; McFarland et al. 1999; Bartlett 2002).

### 3.2 C. difficile virulence factors

The pathogenesis of PMC is mediated by two potent, heat-labile cytotoxic toxins produced by *C. difficile*: toxin A, which is an enterotoxin, and toxin B, a cytotoxin (Lyerly et al. 1988; Pothoulakis and Lamont 2001). Toxin A has a molecular mass of 308 kDa whereas toxin B has a molecular of 279 kDa (Barroso et al. 1990; Dove et al. 1990). The amino acid sequences of the toxins show a high level of homology (von Eichel-Streiber et al. 1990, 1992).

Both toxins are capable of inflicting significant damage to the human colonic epithelium, including modulating fluid secretion and inducing a necrotic inflammatory response, and they act synergistically in this (Savidge et al. 2003). A large range of clinical presentations have been reported, from an asymptomatic carriage to fulminant colitis, depending on the potential overgrowth of the organism. Recent work has revealed the cellular mechanism of action of the toxins (see Figure 1). Both toxins A and B have monoglycosyltransferase activity, which catalyses the incorporation of glucose into a variety of substrate proteins (Just et al. 1995a, 1995b, 1995c). These include the small GTP-binding proteins (Rho, Rac and Cdc42Hs) that are involved in the regulation of the actin cytoskeleton, specifically in the formation of actin stress fibres and focal adhesions. In the diseased state, the colonic epithelium is the major target of *C. difficile* toxins. They cause disruption of the barrier function by opening the tight junctions. This effect is not only caused by the breakdown of actin filaments, but also by inactivation of Rho’s ability to regulate tight junction complexes. These barrier-disrupting effects of toxins A and B increase the colonic permeability, the basis of watery diarrhoea, which is a typical feature of *C. difficile* AAD (Poxton et al. 2001). The colitis is characterised by a massive influx of neutrophils into the colonic mucosa, and in PMC there is an acute inflammatory infiltrate with microabscesses and pseudomembranes rich in neutrophils (Souza et al. 1997).

### 3.3 Pathogenesis of alterations in the function of the intestinal flora

The normal production of lactic acid and short-chain fatty acids (acetate, butyrate, propionate) by the anaerobic flora in AAD is decreased due to diminished digestion of carbohydrates, which results in functional disturbances of the colonic mucosa (Hove et al. 1996; Hogenauer et al. 1998). Moreover, diminished or suppressed carbohydrate metabolism may result in osmotic diarrhoea (‘overload mechanism’) and poor absorption of short-chain fatty acids (‘underload mechanism’; Clausen et al. 1991; Gustafsson et al. 1998), water and electrolytes (cations bound by anionic organic acids; Hammer et al. 1990). Decreased bile acid dehydroxylation, a process that is performed normally by bacteria in the colon (Takamine and Imamura 1995), has been also advocated among the metabolic disturbances resulting from antibiotic use (Hofmann 1977).

### 3.4 Complications

One of the most frequent complications in hospital patients with *C. difficile*-associated diarrhoea is the frequency of relapse (McFarland et al. 1999). This recurrent form of AAD leads to increased use of antibiotics (vancomycin), extended hospital stays and medical complications. *C. difficile*-associated disease (CDAD) causes death in 1–2% of affected patients, whereas the mortality rate increases to 6–30% when PMC is present (Miller et al. 2002; Aslam et al. 2005).

### 3.5 Epidemiology and risk factors

Two main types of predisposing factors have been recognised in AAD: the class of antibiotic administered, and host factors (age and underlying pathologies). Other environmental circumstances may also contribute towards the spread of PMC.

#### 3.5.1 Antibiotic class

Nearly all antibiotics have been reported to be associated with AAD and CDAD, provided that antibiotic concentrations are high enough in the intestinal lumen to inhibit the anaerobes (Wistrom et al. 2001). The most commonly implicated are clindamycin, aminopenicillins (ampicillin/amoxicillin), a combination of amoxicillin and clavulanic acid, second or third generations of cephalosporins (cefuroxime, cefotaxime, cefazidime, ceftriaxone) and, more recently, fluoroquinolones (Johnson et al. 1999; Wistrom...
et al. 2001; McCusker et al. 2003; Loo et al. 2005; Muto et al. 2005; Pepin et al. 2005b). In hospitals, the incidence of AAD is highest in intensive care unit (ICU) patients, with the duration of use of the inciting antibiotic therapy an additional risk factor (Wistrom et al. 2001). In addition, proton pump inhibitors have been implicated as a further possible risk factor (Cunningham et al. 2003; Dial et al. 2004, 2005, 2006; Yearsley et al. 2006).

3.5.2 Host factors

ICU patients with severe underlying pathologies, such as elderly or immunosuppressed patients, or those that have undergone surgical (transplant, gastrointestinal) procedures or that have nasogastric feed tubing, show the highest incidence of AAD and PMC (Brown et al. 1990). While approximately 70% of the population have serum antibodies against toxin A and/or B, the presence of basal levels of antibodies in inpatients exposed to C. difficile is not protective against colonisation (Kyne et al. 2000; Johal et al. 2004). Patient age is one risk factor, with a higher incidence of colonisation at the extreme ages of life (<6 and >65 years old: Brown et al. 1990; Ackermann et al. 2005).

3.5.3 Environmental factors

A hospital offers multiple opportunities for gastrointestinal, nosocomial infections, and the spread of C. difficile between patients has been well-documented (Wistrom et al. 2001). C. difficile is considered a nosocomial organism (Bartlett 2002) and gut surgery and gastrointestinal exploratory procedures increase the risk of AAD (Ackermann et al. 2005).

3.6 Treatments of AAD

3.6.1 Mild or moderate cases of AAD

Asymptomatic carriers of C. difficile should not be treated (Johnson et al. 1992). Treatments for AAD comprise conventional measures such as rehydration and discontinuation of the inciting agent, or replacement of the latter if necessary by a more appropriate antibiotic.

3.6.2 Antibiotic therapy in severe AAD: treatment of PMC

Severe cases of AAD related to C. difficile require a suitably adapted oral antibiotic therapy. The recommended
antibacterial agents are metronidazole, which constitutes first-line therapy (Gerding 2005; Modena et al. 2006), and vancomycin (Teasley et al. 1983). Agents used less frequently include bacitracin (Young et al. 1985), teicoplanin and fusidic acid (Wenisch et al. 1996; Wullt and Odenholt 2004). However, relapses have been reported in all cases (Fernandez et al. 2004; Musher et al. 2005; Pepin et al. 2005a). Moreover, there is concern regarding the selection of vancomycin-resistant organisms, mainly Enterococcus faecium, which is a promiscuous microorganism with respect to the transfer of antibiotic resistance (ASHP 1998).

4. Changes in C. difficile epidemiology

Historically, low rates of severe disease and death (≤3%) may have led to an underestimation of the importance of CDAD as an infection linked to health care (Rubin et al. 1995). Nevertheless, each case of CDAD has been estimated to result in more than $3600 of health care costs, and these costs exceeded $1 billion in the US in 2002 (Kyne et al. 2002). Both the rate and the severity of CDAD may be increasing in US health care facilities. An analysis of data from the National Nosocomial Infections Surveillance system identified an increase in rates from the late 1980s through to 2001 (Archibald et al. 2004).

The epidemiology of C. difficile is changing, as documented in many recently published articles (Pepin et al. 2004). Researchers from the US and Canada note increased rates of CDAD and episodes of more serious disease, both in hospitals and in the community (Pepin et al. 2004). The risk factors for CDAD may also be changing. As noted recently in Europe, North American researchers are also describing a new epidemic strain of C. difficile named BI/NAP1, which is positive for binary toxin and carries the virulence properties and antibiotic resistance patterns of the European strain (ribotype) O27. Current BI/NAP1 strains are more resistant to fluoroquinolones than are historic isolates (McDonald et al. 2005). Canadian researchers also documented it as the predominant strain in a multi-institutional outbreak in the Quebec area. In a prospective study of CDAD in 1703 patients at 12 hospitals in Quebec, Loo et al. (2005) linked the epidemic strain to an increased incidence of C. difficile from 5–6 cases per 1000 admissions to about 25 per 1000 admissions. They also reported that the 30 day mortality rate attributable to infection was 6.9%. The strain was also resistant to fluoroquinolones (Loo et al. 2005).

Community-based cases are also changing. Recently, in the Morbidity and Mortality Weekly Report, four state health departments in the US reported on 33 unusual cases of community-based CDAD that had occurred between 2003 and the middle of 2005 (Anon. 2005). The cases were unusual as disease was severe and involved peripartum women and healthy individuals who had not been recently hospitalised, including eight who had no history of recent antibiotic use.

5. Rationale for living microorganisms (probiotics) in the treatment or prevention of AAD

The human normal intestinal flora is formed of up to 10^{12} bacteria per gram of intestinal content and consists of more than 1000 species (Berg 1996; Suau et al. 1999; Vaughan et al. 2000; Hughes et al. 2001; Blaut et al. 2002; Guerner and Malagelada 2003; Xu and Gordon 2003). Aerobic, facultative and anaerobic bacteria inhabit the gastrointestinal tract. The proportion of anaerobic bacteria increases from the proximal to distal regions, and 99% of inhabitants located in the large intestine are anaerobes. Despite the complexity of the gut bacterial population, its gross composition is remarkably stable and tends to be characteristic for each individual (Zoetendal et al. 1998, 2001; Mai et al. 2004; Vanhoute et al. 2004). All of these bacteria form a stable ecosystem together with the intestinal mucosa (Guerner and Malagelada 2003). The equilibrium of the ecosystem is affected by specific niches for individual bacterial populations, and cooperation in terms of the metabolism of various substrates and cross-feeding between bacteria (Belenguer et al. 2006). Therefore, one important property of this stable ecosystem is colonisation resistance, which permits the elimination of exogenous microorganisms. However, any antibiotic therapy may alter the normal equilibrium of the intestinal microflora, hence encouraging the potential for pathogenic organisms to emerge and cause abnormal growth of C. difficile (Johnson et al. 1999; Wistrom et al. 2001; Pepin et al. 2005b). The hypothesis that living microorganisms could be administered for treatment or prevention of AAD has been supported by the administration of several ‘physiological’ non-pathogenic organisms (Table 1). These have been designated ‘probiotics’ (Cremonini et al. 2002b; Szajewska et al. 2006) or ‘biotherapeutic agents’ (Elmer et al. 1996; Roffe 1996), and defined as “living microbial supplements that exert a beneficial effect on the host by improving the intestinal ecosystem”. Most of these probiotics are bacteria or yeasts used clinically in lyophilised form, and are commercially available.

5.1 Probiotics in the prevention and treatment of AAD

Both bacteria and yeast have been used in the treatment and prevention of AAD, as well as the prevention of associated diarrhoea and reduced relapse (Table 1).

Lactobacillus rhamnosus GG was administered in the form of a yoghurt for 1 week prior to administration of
Table 1. Examples of clinical trials using living microorganisms in the treatment and prevention of antibiotic-associated diarrhoea (AAD)

<table>
<thead>
<tr>
<th>Biotherapeutic agent</th>
<th>Indications</th>
<th>Type of study</th>
<th>No. patients</th>
<th>Dose (per day) and duration of treatment</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em> GG</td>
<td>Treatment of AAD</td>
<td>RPC</td>
<td>16</td>
<td>–</td>
<td>Shortened duration of diarrhoea (8 days)</td>
<td>(Siitonen et al. 1990)</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> + <em>Lactobacillus bulgaricus</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>38</td>
<td>20.4 × 10⁸ CFU/day, for 10 days (min 5 days)</td>
<td>No significant prevention in the treated group</td>
<td>(Tankanow et al. 1990)</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em></td>
<td>Prevention of AAD</td>
<td>RPC</td>
<td>10</td>
<td>3 yoghurts/day, for 3 days</td>
<td>No significant increase in weight and frequency of stools in treated group vs. placebo</td>
<td>(Colombel et al. 1987)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>188</td>
<td>1 × 10¹⁰ CFU to 2 × 10¹⁰ CFU/day, for 10 days</td>
<td>17% AAD vs. 48% in placebo group</td>
<td>(Young et al. 1998)</td>
</tr>
<tr>
<td><em>Saccharomyces boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>388</td>
<td>4 capsules/day, variable duration of treatment (min 5 days)</td>
<td>4.5% AAD vs. 17.5% in placebo group</td>
<td>(Adam et al. 1977)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>180</td>
<td>1 g/day, variable duration of treatment</td>
<td>9.5% AAD vs. 14.6% in placebo group</td>
<td>(Surawicz et al. 1995)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>72</td>
<td>113 mg twice a day, for 49 days</td>
<td>No significant prevention in the treated group</td>
<td>(Lewis et al. 1998)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>193</td>
<td>1 g/day, for 49 days</td>
<td>7.2% AAD vs. 14.6% in placebo group</td>
<td>(McFarland et al. 1995)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>269</td>
<td>500 mg/day, for the duration of antibiotic treatment (experimental group 7.8±1 day; control group 8.1±1 day)</td>
<td>3.4% AAD vs. 17.3% in placebo group</td>
<td>(Kotowska et al. 2005)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>151</td>
<td>500 mg/day, for the duration of antibiotic treatment</td>
<td>1.4% AAD vs. 9% in placebo group (p &lt; 0.05)</td>
<td>(Can et al. 2006)</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Biotherapeutic agent</th>
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</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em> + <em>Bifidobacterium bifidum</em></td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>150</td>
<td>$2 \times 10^{10}$ CFU each strain/day, for 20 days</td>
<td>2.9% toxins vs. 7.3% in placebo group</td>
<td>(Plummer et al. 2004)</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> SF68</td>
<td>Prevention of AAD</td>
<td>DBPC</td>
<td>45</td>
<td>$7.5 \times 10^7$ CFU/day, for 7 days</td>
<td>9% AAD vs. 27% in placebo group</td>
<td>(Wunderlich et al. 1989)</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG</td>
<td>Prevention of relapses of PMC</td>
<td>Open</td>
<td>5</td>
<td>–</td>
<td>No relapses in four patients, metronidazole in one patient</td>
<td>(Gorbach et al. 1987)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of relapses of PMC</td>
<td>DBPC</td>
<td>124</td>
<td>1 g/day, for 28 days</td>
<td>Further relapses in 26% of treated patients vs. 45% in placebo group</td>
<td>(McFarland et al. 1994)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of relapses of CDAD</td>
<td>Open</td>
<td>19</td>
<td>–</td>
<td>Diminished number of relapses</td>
<td>(Buts et al. 1993)</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299v</td>
<td>Prevention of relapses of CDAD</td>
<td>DBPC</td>
<td>20</td>
<td>$5 \times 10^{10}$ CFU/day, for 38 days</td>
<td>Recurrence rate 36% vs. 67% in placebo group</td>
<td>(Wullt et al. 2003)</td>
</tr>
<tr>
<td><em>L. plantarum</em> 299v</td>
<td>Reduction of the negative effects of an antibiotic on colonic fermentation</td>
<td>DBPC</td>
<td>19</td>
<td>–</td>
<td>Significant decrease in total SCFA in the placebo group ($p = 0.028$) but not in the <em>Lactobacillus</em> group</td>
<td>(Wullt et al. 2007)</td>
</tr>
<tr>
<td><em>S. boulardii</em></td>
<td>Prevention of relapses of CDAD</td>
<td>DBPC</td>
<td>209</td>
<td>1 g/day, for 28 days</td>
<td>Recurrence rate 17% vs. 50% in placebo group with high-dose vancomycin. No difference in recurrence rate in treated group vs. placebo group with low-dose vancomycin or metronidazole</td>
<td>(Surawicz et al. 2000)</td>
</tr>
</tbody>
</table>

CDAD: *Clostridium difficile*-associated diarrhoea; DBPC: double blind placebo controlled; PMC: pseudomembranous colitis; RPC: randomized placebo controlled; SCFA: short-chain fatty acids; -: data not available
erythromycin (400 mg three times/day). This resulted in a diminished duration of diarrhoea in 16 patients compared to a placebo group (2 vs. 8 days; \( p < 0.05 \)) and \( C. \) difficile toxin was negative in all 16 patients at the end of the treatment period (Sitonen et al. 1990). In 38 paediatric patients with amoxicillin-induced diarrhoea the administration of a mixture of \textit{Lactobacillus acidophilus} and \textit{Lactobacillus bulgaricus}, four times a day for 10 days together with the antibiotic therapy, had no preventative effect on diarrhoea (Tankanow et al. 1990). Stool samples were not tested for \textit{C. difficile} (culture and/or toxin). However, in a randomised placebo-controlled cross-over study in 10 healthy volunteers receiving erythromycin (3 days, 1 g twice/day), oral ingestion of yoghurt containing \textit{Bifidobacterium bifidum} significantly diminished the number of stools (double in the placebo group but unchanged in the yoghurt group) and degree of abdominal pain compared to volunteers receiving placebo-yoghurt (Colombel et al. 1987). Stool samples were not tested for \textit{C. difficile} (culture and/or toxin). In 188 children (6 months to 10 years old) treated with antibiotics, administration of \textit{Lactobacillus} GG (1–2 capsules a day, \( 10^{10} \) CFU per capsule) resulted in a significant difference in the occurrence of diarrhoea (16 out of 93 children) compared to the placebo group (46 out of 95 children, \( p < 0.0001 \); Young et al. 1998). In a recent double-blind, randomised, placebo-controlled trial, 150 elderly patients received standard antibiotic treatment plus \( 2 \times 10^{10} \) CFU \textit{L. acidophilus} and \textit{Bifidobacterium bifidum}/capsule per day for 20 days. On the basis of the development of diarrhoea, the incidence of samples positive for \textit{C. difficile}-associated toxins was 2.9% in the probiotic group vs. 7.25% in the placebo group. When samples from all patients were tested, 46% of probiotic patients were toxin-positive compared with 78% of the placebo group (Plummer et al. 2004).

\textit{E. faecium} SF68 has shown only modest efficacy in the prevention of AAD in two controlled clinical trials. Forty-five patients being treated with antibiotics were given, concurrently, one capsule twice daily of either \textit{E. faecium} SF68 (7.5 × 10⁷ CFU) or placebo for 7 days. The rate of AAD was 9% with SF68 compared with a placebo rate of 27% (Wunderlich et al. 1980). In the second study (not double-blind), 200 patients received antituberculosis antibiotics. Patients administered orally with \textit{E. faecium} SF68 showed a lower rate (5%) of AAD compared to the placebo group (18%; Borgia et al. 1982).

In 388 ambulatory patients with upper respiratory tract infections receiving tetracycline or \( \beta \)-lactams for longer than 5 days, \textit{Saccharomyces boulardii} reduced the prevalence of AAD to 4.5% compared to 17.5% in the placebo group (\( p < 0.001 \); Adam et al. 1977). Stool samples were not tested for \textit{C. difficile} (culture and/or toxin). In a double-blind, placebo-controlled study with 180 patients receiving any antibiotic except vancomycin or metronida-
reported (Pochapin 2000). The recurrence rate was similar for the probiotic (36.4%) and placebo groups (35.7%). A small study of 20 patients with recurrent CDAD, metronidazole and either Lactobacillus plantarum 299v (5 × 10^{10} CFU/day) or placebo were given for 38 days (Wullt et al. 2003). Although the recurrence rate was lower in the L. plantarum group (36.4%) compared to the placebo (66.7%), the difference was not significant. In 19 children with persistent diarrhoea associated with the presence of C. difficile in stools, S. boulardii administered as a monotherapy resulted in a significant improvement in symptoms in 18 patients after 8 days of treatment (Buts et al. 1993). In a double-blind, randomised, controlled study, S. boulardii versus placebo was given to 124 patients with recurrent CDAD. The number of relapses was significantly reduced to 26% compared to the placebo group at 45% (p = 0.05). The efficacy of S. boulardii in preventing relapses has been shown more clearly in patients who had a previous relapse of AAD. In these patients, relapses occurred in 35 vs. 65% (p = 0.04) in the placebo control (McFarland et al. 1994). In an effort to further refine a standard regimen, the same group tested patients receiving a standard antibiotic for 10 days and then added either S. boulardii (1 g/day for 28 days) or placebo. A significant decrease in the recurrence was observed only in patients treated with high-dose vancomycin (2 g/day) and S. boulardii (17%), compared to those who received high-dose vancomycin and placebo (50%, p = 0.05). However, S. boulardii treatment had no impact on the recurrence rates in patients treated with a low dose of vancomycin or metronidazole (Surawicz et al. 2000).

A recent clinical trial demonstrated that administration of L. plantarum 299v reduced the negative effects of metronidazole on colonic fermentation (Wullt et al. 2007). The authors suggested that intake of L. plantarum 299v affected concentrations of faecal organic acids during and after metronidazole treatment in 19 patients with recurrent C. difficile-associated diarrhoea. Following the intake of metronidazole, a significant decrease in total short-chain fatty acids was seen in the placebo group (from 77.1 to 45.5 µmol/g, p = 0.028), but no effect was seen in the Lactobacillus group (79.8 to 60.4 µmol/g). In addition, a statistically significant difference between treatment groups was noted for butyrate (from 5.6 to 1.2 µmol/g in the placebo group vs. 7.6 to 5.6 µmol/g in the Lactobacillus group, p = 0.047). At the end of the study and after cessation of placebo or Lactobacillus treatment, total short-chain fatty acids rose to the same levels as those recorded prior to the start of antibiotic treatment in the placebo group. Therefore it is possible that intake of this probiotic strain may provide an additional benefit for patients with recurrent C. difficile-associated diarrhoea.

6. Conclusions

AAD and C. difficile are common clinical problems. The concept of replacing disease-inducing pathogenic organisms with non-pathogenic ones appears useful. The use of probiotics in the control of gastrointestinal disorders offers an alternative approach/adjunct to conventional antibiotic therapies. Several live microorganisms have been used in the treatment and prevention of AAD and CDAD. They include L. rhamnosus GG, B. bifidum or yeasts such as S. boulardii. The results discussed here show an overall reduction in the risk of AAD during probiotic administration. The most reproducible results so far have been achieved with S. boulardii and L. rhamnosus GG predominantly, both in terms of reducing the incidence of AAD and the number of relapses in recurrent C. difficile-associated diarrhoea. However, the number of clinical trials with ‘biotherapeutic’ agents is limited, and one of the main issues is inconsistency; many studies on AAD have not tested for C. difficile (culture and/or toxins) before or after treatment, the definition of diarrhoea within the studies varies, the duration and the dose of treatment varies as does the follow-up period, and the number of patients and the design in many (early) studies is considered inadequate. Some theoretical considerations also arise regarding the possibility of side-effects with ‘biotherapeutic’ agents such as bacteremia, especially in immunosuppressed patients. These concerns have, however, not been confirmed by clinical experience. Therefore, further well-designed, controlled clinical trials with standard definitions and fixed parameters need to be undertaken, using various probiotic preparations for the treatment of confirmed C. difficile diarrhoea. In conclusion, it would appear that there is some merit in the probiotic approach to address C. difficile-induced problems. Certainly, there is a need to address the ubiquity and consequences of this pathogen.

7. References


Dietary-based gut flora modulation against Clostridium difficile onsets C. Gougoulas et al. 39


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