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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.

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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.* 1998; 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, ceftazidime, ceftriaxone) and, more recently, fluoroquinolones (Johnson *et al.* 1999; Wistrom

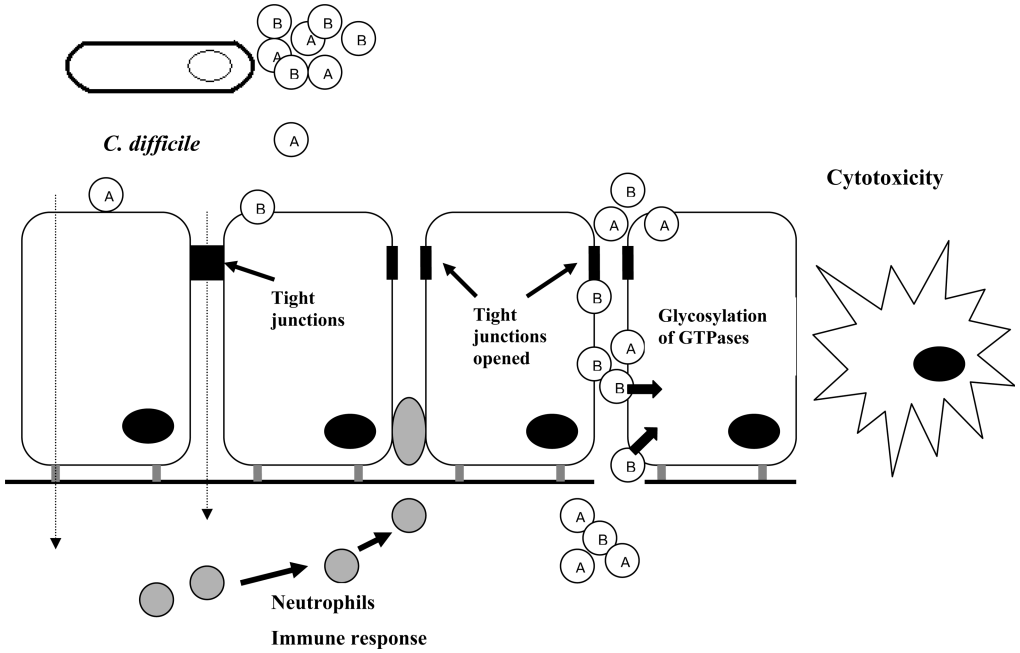

Figure 1. Mechanism of action of *Clostridium difficile* toxins A and B on the intestinal epithelium. Both toxins modify small proteins (GTP-binding proteins) that regulate the actin cytoskeleton by incorporating a glucose molecule into them. The toxins then cause disruption of the barrier function of the intestinal epithelium by opening the tight junctions and increasing colonic permeability. This is because GTPases regulate tight junction complexes. The immune response is characterised by a massive influx of neutrophils. Reproduced with permission from Ian R. Poxton, University of Edinburgh, UK.

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 ($\leq$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 (ribotybe) 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 fluoroquinolines (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; Guarner 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 (Guarner 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)

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

(*Continued*)

Table 1. (*Continued*)

Biotherapeutic agent	Indications	Type of study	No. patients	Dose (per day)and duration of treatment	Results	Reference
L. acidophilus + *Bifidobacterium bifidum*	Prevention of AAD	DBPC	150	2×10^{10} CFU each strain/day, for 20 days	2.9% toxins vs. 7.3% in placebo group	(Plummer *et al*. 2004)
Enterococcus faecium SF68	Prevention of AAD	DBPC	45	7.5×10^{7} CFU/day, for 7 days	9% AAD vs. 27% in placebo group	(Wunderlich *et al*. 1989)
L. rhamnosus GG	Prevention of relapses of PMC	Open	5	–	No relapses in four patients, metronidazole in one patient	(Gorbach *et al*. 1987)
S. boulardii	Prevention of relapses of PMC	DBPC	124	1 g/day, for 28 days	Further relapses in 26% of treated patients vs. 45% in placebo group	(McFarland *et al*. 1994)
S. boulardii	Prevention of relapses of CDAD	Open	19	–	Diminished number of relapses	(Buts *et al*. 1993)
Lactobacillus plantarum 299v	Prevention of relapses of CDAD	DBPC	20	5×10^{10} CFU/day, for 38 days	Recurrence rate 36% vs. 67% in placebo group	(Wullt *et al*. 2003)
L. plantarum 299v	Reduction of the negative effects of an antibiotic on colonic fermentation	DBPC	19	–	Significant decrease in total SCFA in the placebo group ($p = 0.028$) but not in the *Lactobacillus* group	(Wullt *et al*. 2007)
S. boulardii	Prevention of relapses of CDAD	DBPC	209	1 g/day, for 28 days	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	(Surawicz *et al*. 2000)

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 (Siitonen *et al.* 1990). In 38 paediatric patients with amoxicillin-induced diarrhoea the administration of a mixture of *Lactobacillus acidophilus* and *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 *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 *Bifidobacterium longum* 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 *C. difficile* (culture and/or toxin). In 188 children (6 months to 10 years old) treated with antibiotics, administration of *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 2×10^{10} CFU *L. acidophilus* and *Bifidobacterium bifidum*/capsule per day for 20 days. On the basis of the development of diarrhoea, the incidence of samples positive for *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).

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 *E. faecium* SF68 (7.5×10^7 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 antituberculic antibiotics. Patients administered orally with *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 β-lactams for longer than 5 days, *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 *C. difficile* (culture and/or toxin). In a double-blind, placebo-controlled study with 180 patients receiving any antibiotic except vancomycin or metronidazole, the occurrence of AAD was limited to 9.5% in patients treated with *S. boulardii* compared to 22% in the placebo group (Surawicz *et al.* 1995). Stool samples were also tested for *C. difficile* (culture and toxin). Similarly, in 193 patients receiving β-lactam antibiotics, the administration of *S. boulardii* (1 g daily for 2 weeks) in addition to antibiotics, resulted in a lower incidence of AAD (7.2% of patients receiving *S. boulardii* compared to 14.6% in the placebo group, $p = 0.02$; McFarland *et al.* 1995). Simultaneous administration is possible because of its resistance to most antibiotics (Bergogne-Berezin 1995). However, Lewis *et al.* (1998) reported a lack of a therapeutic effect with *S. boulardii* (113 mg twice daily) in the prevention of AAD in a placebo-controlled study with 72 elderly patients. The lack of effect was attributed to the low dose used. However, in a recent double-blind, randomised placebo-controlled trial of 269 children (aged 6 months to 14 years) with otitis and/or respiratory tract infections, standard antibiotic treatment together with 250 mg of *S. boulardii* orally twice daily resulted in a lower prevalence of diarrhoea than in those receiving antibiotic plus placebo (8 vs. 23%). In a separate study, the occurrence of AAD was also limited to 3.4% in patients treated with *S. boulardii* compared to 17.3% in the placebo group (Kotowska *et al.* 2005). One hundred and fifty-one patients hospitalised at the Gulhane Military Medical Academy's Department of Infectious Diseases and Clinical Microbiology were given *S. boulardii* twice daily during the course of antibiotic therapy. Patients did not require intensive care therapy and application was initiated as late as 48 h after antibiotic therapy. The rate of development of AAD was found to be 9% (7/78) in the placebo group was and 1.4% (1/73) in the study group ($p < 0.05$). Stool samples from the patients with AAD were assayed for *C. difficile* toxin A as late as 7 days after the collection of samples. The assay for *C. difficile* toxin A yielded positive results in two stool samples from the seven patients with AAD in the placebo group and a negative result was obtained for the one patient who developed AAD in the study group. The results implied that prophylactic use of *S. boulardii* in hospitalised patients resulted in a reduced incidence of AAD, with no serious side effects seen (Can *et al.* 2006).

5.2 Probiotics in the prevention and treatment of CDAD

Early uncontrolled trials of *Lactobacillus rhamnosus* GG suggested a possible efficacy in recurrent CDAD. In four different studies, 8 out of 11 adults (Gorbach *et al.* 1987), two out of four children (Biller *et al.* 1995), five out of nine adults (Bennett *et al.* 1990) and 27 out of 32 adults (Bennett *et al.* 1996) demonstrated beneficial effects.

A single randomised, controlled trial using *L. rhamnosus* GG with either metronidazole or vancomycin has been

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 *C. difficile* associated-diarrhoea, with a view of preventing relapses. 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 bacteraemia, 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

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