Review

Saccharomyces boulardii: A Review of an Innovative Biotherapeutic Agent

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***Saccharomyces boulardii* is a non-pathogenic yeast which has been used as both a preventive and therapeutic agent for the treatment of a variety of diarrheal diseases. The studies with animal models and evidence from human volunteers and patients indicate a profile which is effective in the therapy of diarrhea and is remarkably safe for oral ingestion. The pharmacokinetic data demonstrate that *S. boulardii* reaches a steady-state concentration quickly and maintains a high stable level as long as the yeast is taken daily. Once the agent is discontinued, *S. boulardii* is quickly eliminated from the colon. Clinical trials studying antibiotic-associated diarrhea, nasogastric-tube alimentation diarrhea, *Clostridium difficile*-disease, acute diarrhea and chronic diarrhea in HIV-infected patients are reviewed.**

KEY WORDS-*Saccharomyces boulardii*; Biotherapeut ic agent; *Clostridium difficile*; Diarrhea.

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INTRODUCTION

Gastrointestinal disease is often a consequence of a myriad of factors which disturb the bowel's complex ecosystem. Antibiotics are the most common culprit of acute diarrhea due to the loss of "colonisation resistance" or the protective role of normal intestinal flora against pathogenic organisms [74]. A great variety of antibiotics have been implicated, but the most frequently associated with diarrhea are penicillins (especially ampicillin or amoxicillin), cephalosporins and clindarnycin [32,42,55,71]. Over one-third of antibiotic-associated diarrhea is associated with an infection by an anaerobic bacterium, *Clostridium difficile*, which also causes nosocomial (hospital-acquired) outbreaks [3,12,51]. In addition, medications and in-hospital procedures have been as sociated with a higher risk of diarrhea in nosocomial outbreaks [52,56]. Host factors such as advanced age, gender and severe underlying disease conditions have been implicated in higher risks of acquiring nosocomial diarrhea [2,52].

Other aetiologies of diarrhea are due to infections not associated with antibiotic predisposition (e.g. toxigenic *Escherichia coli* and *Vibrio cholerae*, or infection with *Entamoeba histolytica*, *Giardia lamblia*, or viruses). In many instances of acute diarrhea in children, hospitalised patients or HIV-infected patients, the aetiological agent has not been determined.

The traditional treatment for acute diarrhea often depends on whether a known aetiological agent can be identified, on the severity of symptoms and the source of the infection (community or nosocomial). Electrolyte replenishment and cessation of the inciting agent (antibiotic or medication) are often all that is required for the treatment of milder forms of diarrhea. Specific therapy may be prescribed if a specific aetiological agent can be detected. Unfortunately, these steps are not always sufficient and the diarrhea may continue and become chronic, symptoms may increase in severity and spectrum, or toxic megacolon or death may ensue [26,32].

In an effort to prevent or treat these difficult cases of diarrhea and to also re-establish the normal homeostasis of the colonic ecosystem, innovative approaches have been tried using living, biotherapeutic agents. The biotherapeutics which have been investigated previously include *Saccharomyces boulardii*, *Lactobacillus species*, *Bifidobacterium bifidum*, or faecal enemas [8,36,46,48,63,69]. This review will focus on *S. boulardii.*

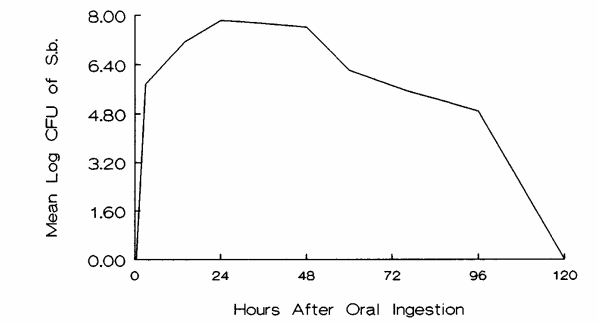

Figure 1: Time of elimination of *S. boulardii* (*S.b.*) in human volunteers given a single oral dose (1 g).

HISTORICAL DEVELOPMENT

S. boulardii (Ultra-Levure™, Perenterol™, Floratil™ was first isolated from lychee fruit in Indochina and used in France to treat diarrhea, beginning in the 1950s. It has an unusually high optimal growth temperature of 37°C. A lyophilised form was marketed by Laboratoires Biocodex (Montrouge, France) commencing in 1962 and mainly used for antibiotic-associated diarrhea. It is widely available in Europe, South America and Africa. In the USA, it is an investigational new drug currently undergoing Food and Drug Administration (FDA) approved phase III clinical trials.

EFFECT ON THE GASTROINTESTINAL TRACT

Pharmacokinetics

The ability to differentiate *S. boulardii* from other colonic flora allows pharmacokinetic studies to be performed to study the behaviour and fate of this living oral biotherapeutic agent. These studies indicate that this yeast is well suited as a treatment agent because it is able to achieve high concentrations in the colon quickly, maintain constant levels, does not permanently colonise the colon and does not translocate easily out of the intestinal tract [4,5,7]. In gnotobiotic mice, a single dose of *S. boulardii* appears to colonise the intestinal tract and yeast is detectable at a constant, albeit low, level (10^7 c.f.u.) for 60d [27]. In healthy human volunteers given a single oral dose of 1 g *S. boulardii* (Figure 1), the time to maximum stool concentration was within 36-60 h, with *S. boulardii* below detectable levels at a range of 2-5 d later [5,44]. In these volunteers, the mean recovery of a single oral dose was 0.12 ± 0.04 per cent determined as viable yeast in the stool [44].

When *S. boulardii* was administered daily (0.8 g/kg, oral) to rats, steady-state concentrations were achieved within a mean of 3 d and a constant level was maintained as long as oral dosing was continued [5]. In volunteers, steady-state elimination was achieved between 72 and 120 h at a range of means between 3.6×10^7-8.6×10^8 c.f.u. when volunteers were given 100 mg to 1.5 g *S. boulardii* twice daily [44]. There was a linear dose recovery observed in humans (Figure 2). Similar to the rat, the mean recovery was 0.2 per cent of the ingested dose recovered as viable yeast in the stools of healthy volunteers.

The concentration of *S. boulardii* in the stool and the percentage recovery were significantly increased if antibiotics which are active against anaerobes were co-administered with the *S. boulardii.* An increase in viable *S. boulardii* was found in healthy human volunteers given ampicillin (0.5 g, BID for 8 d) with S. boulardii (1-3 g/d). The recovery of viable *S. boulardii* rose from 0.20 per cent when no ampicillin wasco-administered to 0.43 percent when ampicillin was given and the maximum concentration went from 2×10^8 c.f.u./g to 6.1×10^8 c.f.u./g after ampicillin [44].

Further research on the pharmacokinetic behaviour of this organism is needed. In addition, studies which examine the influence of diet, other antibiotics or medications and other factors which affect the gastrointestinal ecology seem warranted.

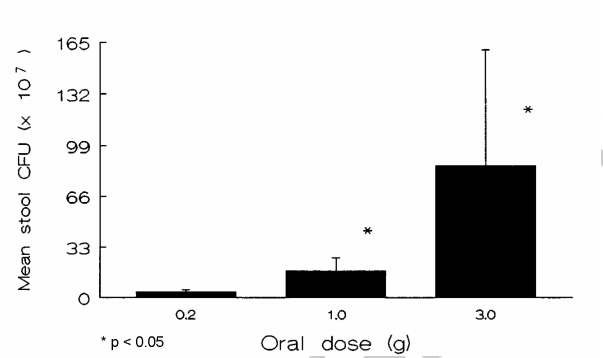

Figure 2. Dose recovery of *S. boulardii* in adult volunteers at steady-state concentrations for three doses (200 mg, 1 g and 3 g). (Reproduced with permission from Klein *et al.* 1993 [44]).

Immunological response

In vitro S. boulardii activates complement directly and fixes C3b. The phagocytosis of *S. boulardii* by mononuclear cells is complement dependent [47].

Oral ingestion of *S. boulardii* causes an increase in secretory IgA and secretory component in the small intestines of rats. Buts and colleagues [10] found that suckling and weaning rats given high doses of *S. boulardii* (0.5 mg/g t.i.d.) had an 80 per cent increase in secretory component in the crypt cells over controls (saline and ovalbumin) and 69 per cent increase in secretory component in villus cells ($P<0.05$). The mean secretory IgA level was increased 56.9 percent in *S. boulardii* treated rats over controls ($P<0.01$) given saline [10].

In human volunteers, given S. boulardii (1 g/d for 7 d), an increase in peripheral blood cells (indicative of an inflammatory process) was observed by Caetano et al [12]. Significant increases in mean number of erythrocytes, haemoglobin, leukocytes, neutrophils, polynuclear cells and phagocytes were observed in 60 human volunteers [47]. No significant increases in the mean number of eosinophils, basophils, lymphocytes, monocytes or platelets were noted. The significance of this observation is unclear as when these blood cell populations were examined in clinical trial patients, no significant changes were noted [67].

Effect on intestinal mucosa

Electron microscopic examination of duodeno-jejunum mucosa in rats given *S. boulardii* (75 mg/d for 5 d) and mice (30 min to 24 h) showed no invasion of *S. boulardii* into the mucosal layers, and no morphological changes of the villi or changes of crypt depth were noted [9,14,21].

Oral administration of *S. boulardii* (l g/d for 14 d) was shown by Buts *et al.* to increase the duodeno-jejunum levels of mucosal sucrase (increase of 82 per cent), lactase (77 percent) and maltase (75 percent) in seven human volunteers [9]. An increase in these three enzymes was also found in rats fed *S. boulardii* (75 mg/d for 5 d) compared with controls [9]. In rats and humans, this increase in disaccharidase activity may improve the absorption of carbohydrates which have been associated with diarrhea [58,59].

MICROBIAL INTERACTIONS

Effect on normal flora

A study on healthy human volunteers given 1 g *S. boulardii*/d showed no significant changes in selected populations of normal colonic flora after 45 d exposure to the yeast [44]. Concentrations of total anaerobes, *Bacteroides* species, or *Clostridium* species did not significantly increase or decrease after 45 d compared with their baseline counts. The mean concentrations of two groups of bacteria slightly increased from pre-*S. boulardii* exposure to post-*S. boulardii*: total aerobes (mean = 1.4 x 10^6/g stool to 2.l x 10^8/g, respectively) and total coliforms (mean = 1.8 x 10^6/g to 1.9 x 10^7/g, respectively) [44].

Specific microbial interactions

In an undisturbed bowel with functioning colonisation resistance, *S. boulardii* can be introduced with no apparent effect on the microflora. When there is an overgrowth of pathogenic organisms, *S. boulardii* has been shown to reduce the concentrations of several aetiological agents of diarrhea or their associated toxins.

Clostridium difficile

C. difficile is a strict anaerobe which produces two well-characterised toxins, and is the most frequent cause of nosocomial diarrhea in adults. Intestinal colonisation by *C. difficile* occurs after colonisation resistance has been compromised by antibiotic use, medications, surgery or gastrointestinal procedures. The incidence of *C. difficile* has ranged from 0.8 to 21 per 100 hospitalised patients receiving antibiotics [40,51].

S. boulardii has been tested in animal models of *C. difficile*-associated colitis and has been found to have a protective effect if challenged with either toxigenic C. difficile or purified toxin A or B (Table 1). Corthier et al. [19] found that gnotobiotic mice, who usually die after a *C. difficile* challenge, were protected after a single dose of *S. boulardii* (16 per cent survival) and 56 per cent survived if the S. boulardii was given continuously.

Table 1. Inhibitory actions of *S. boulardii* on various aetiological agents of diarrhea in animal models

Aetiological agent	Animal tested	Factor model	Result in *S. boulardii* treated animals	Result in control animals	Reference cited
Clostridium difficile (CD)	Gnotobiotic mice	Survival	56%	0%	Corthier *et al*, 1986 [19]
	Hamsters	Survival	100%	28%	Elmer and McFarland 1987 [29]
	Hamsters	c.f.u. CD/g	$\log_{10}$ 5.7	$\log_{10}$ 8.8	Elmer and McFarland, 1987 [29]
	Hamsters	Cytotoxin	3%	51%	Elmer and McFarland, 1987 [29]
	Mice	Cytotoxin (ng/g)	$\log_{10}$ 0.9	$\log_{10}$ 3.3	Castex *et al*, 1990 [16]
	Rabbit brush borders	Enterotoxin binding	38%	100%	Pothoulakis *et al.*, 1993 [57]
	Rat ileal loops	weight (mg/cm)	200-250	320	Pothoulakis *et al.*, 1993 [57]
Candida albicans	Immunosuppres sed mice	Translocation *C. albicans*+ lymph nodes	53%	72%	Berg *et al.* 1993 [4]
	Gnotobiotic mice	c.f.u. *C. albicans*	$\log_{10}$ 6.30	$\log_{10}$ 7.5	Ducluzeau and Bensaada, 1982 [27]
Vibrio cholerae	Rat intestinal loops	Secretion (ml/20min/10cm)	0.25	0.43	Vidon *et al.*, 1986 [75]
	Rat epithelial cell line	cAMP level (pmol/mg)	1300	2700	Czerucka *et al.*, 1989 [22]
Escherichia Coli	Infant mice	Intestinal weight/body weight ratio	0.076	0.101	Massot *et al*, 1983 [49]
Entamoeba histolytica	Rats	Infection severity > 1	32%	91%	Rigothier *et al.*, 1990 [60]

This protection from *C. difficile*-induced mortality was later found to be dependent upon the dose and viability of the yeast [31]. The ability of *S. boulardii* to inhibit *C. difficile*-associated damage is lost if the yeast is given in a non-viable state (killed by heat or amphotericin B). In addition, a dose response was observed in a study by Elmer and Corthier [31]. As the dose of S. boulardii was increased from 3 x 108/ml to 3.3 x 1010/ml in drinking water given to mice, the survival increased linearly from 0 per cent to 85 per cent [31]. In Syrian hamsters, S. boulardii was found to reduce clindamycin-induced mortality which results from *C. difficile* infection [29,50,73]. Several studies found *S. boulardii* reduced the levels of *C. difficile* in hamster faecal pellets, but different findings on the influence of *S. boulardii* on the toxins of *C. difficile* have been reported [29,50].

While *S. boulardii* has been reported to decrease levels of *C. difficile* in faecal pellet samples in the gnotobiotic mouse and hamster models, the most prominent effect of *S. boulardii* treatment is the decrease in concentrations of *C. difficile* toxins A and B [20,29,31].

S. boulardii failed to have a significant impact on cytotoxin levels *in vitro*, but was protective in gnotobiotic mice, and other studies in mice or hamsters have shown a decrease in toxin A and/or cytotoxin after *S. boulardii* exposure [15,21,29]. Czerucka *et al.* [23] found a decrease in the percentage rounding of intestinal cells due to *C. difficile* if *S. boulardii* was added to the cell culture, but another study failed to show this protective effect [57].

Early studies with histological sections or electron microscopy showed that *S. boulardii* inhibited the formation of the typical lesions associated with the toxins of *C. difficile* in mice and hamsters [15,16,21,50] As shown in Figure 3, the caecal mucosa of mice given *C. difficile* was degraded whereas the caecal mucosa of mice fed *C. difficile* with *S. boulardii* was undamaged. Pothoulakis *et al.* reported a protein with protease activity produced by *S. boulardii* which may be responsible for the lessening of the *C. difficile* toxins' effect on intestinal mucosa [57]. The large exogenous protein is retained by a 100 000 MW filter and decreases fluid secretion in a rat loop model, but appears to have no effect on cell tissue layer damage caused by *C. difficile* when using human lung (IMR-90) fibroblasts or rat basophilic leukaemia (RBL) cell lines. The proteinase-like product inhibited purified ^{3}H-labelled enterotoxin-binding to intestinal rabbit ileal brush borders by 37 per cent, reduced enterotoxin induced fluid secretion by 55 per cent in rat ileal loops and reduced mannitol permeability by 93 per cent in rat ileal loops [57]. Furthermore, when *S. boulardii* was given orally for 3 d to rats, a challenge by pure enterotoxin failed to increase fluid secretion or permeability. In contrast, when *S. boulardii* and enterotoxin were co-administered, no protective effect of *S. boulardii* was observed. Thus *S. boulardii* may not have a direct action on the toxins of *C. difficile. S. boulardii* may reduce the toxin-mediated effects of C. difficile diarrhea through its protease-like product which degrades specific receptor sites on the intestinal mucosa [57].

Candida albicans

The role of *C. albicans* in diarrhea is a debated issue, yet its role as an opportunistic pathogen is clear especially in immunocompromised hosts [45, 76]. In immunosuppressed animal models and in humans, *C. albicans* has the ability to translocate from the bowel to other locations in the body. Berg *et al.* [4] tested *S. boulardii* in order to determine if the yeast could inhibit this phenomenon. When antibiotic-decontaminated and immunosuppressed mice were treated with *S. boulardii* (5 per cent in drinking water) per day for 9 d, significantly fewer (53 per cent) of the mesenteric lymph nodes or spleens examined had *C. albicans* compared with 72 per cent (P=0.02) of the mesenteric lymph nodes of the control mice.

A study by Ducluzeau and Bensaada [27] showed that concentrations of $\geq 10^{9}$ c.f.u. of *S. boulardii*/g of stool inhibited the concentration of *C. albicans* by 10-50 per cent in gnotobiotic mice. *S. boulardii* also inhibited the *in vivo* proliferation of *C. krusei* and *C. pseudotropicalis* but had no inhibitory action on *C. tropicalis*.

Cholera

Vibrio cholerae produces a toxin which activates adenylate cyclase of the enterocyte and stimulates cAMP production resulting in profound watery diarrhea. In an early study by Vidon *et al.*, [75] intestinal loops were surgically created in male Wistar rats and injected with either a 2 h pre-incubated mixture of *S. boulardii* ($3x10^9$ c.f.u./ml) and cholera toxin (10 µg/ml) or a mixture of cholera toxin and buffer (control loops). *S. boulardii* inhibited approximately 50 per cent of the fluid and sodium secretion compared with loops treated with cholera toxin alone. Irradiated or heat-killed *S. boulardii* preparations were found to have an inhibitory effect when co-administered with cholera toxin though *S. boulardii* given alone had no effect on water secretion in the jejunal loops.

The inhibitory action on cholera toxin was confirmed by Czerucka *et al.* [22] using rat epithelial intestinal cell lines. The cell lines were pre-incubated with *S. boulardii* for 48 h and then exposed for 90 min to 1 µg/mm cholera toxin. The amount of cAMP was decreased by 50 per cent in viable *S. boulardii*-treated cell lines compared with control cell lines (cholera toxin only). In contrast to the study by Vidon *et al*. [75], this study found if heat-killed *S. boulardii* was given, the level of cAMP was similar to the control cell lines.

Current studies show that *S. boulardii* secretes a protein which decreases the concentration of cholera toxin-induced cAMP in epithelial cell lines [24, 25]. Czerucka hypothesises that *S. boulardii* may act on cells through a receptor coupled to a pertussis toxin-sensitive G protein [25].

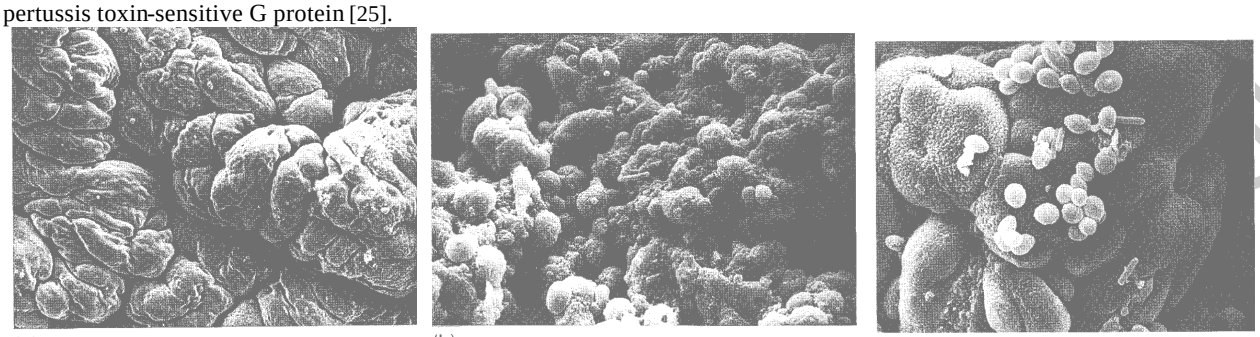

Figure 3. Scanning electron photomicrographs of caecal mucosa of axenic mice. **a**, Normal caecal mucosa (SEM x 417). **b**, Caecal mucosa of mice challenged with toxigenic *C. difficile* (SEM x 1990). **c**, Caecal mucosa treated with *S. boulardii* and challenged with toxigenic *C. difficile* (SEM x 2600). (Reproduced with permission from F. Castex, S. Jouvert and M. Bastide, Laboratory of Immunology and Virology, Montpellier, France)

Escherichia coli

Massot *et al.* [48] studied an enterotoxic strain of *E. coli* (K88) in infant mice which respond only to the thermostable (ST) enterotoxin. Mice were injected with 500 mg/kg *S. boulardii* and *E. coli* and sacrificed 4 h later.

Animals receiving *E. coli* alone had a mean intestinal weight/body weight ratio of 0.101 compared with mice treated with *E. coli* and *S. boulardii* when the ratio was reduced to a mean of 0-076. Killed *S. boulardii* cells had no effect on the inhibition of *E. coli*-induced fluid secretion.

Entamoeba histolytica

S. boulardii reduced the number and severity of lesions caused by *E. histolylica* in young rats in a study by Rigothier *et al.* [60] CDR Sprague-Dawley young rats were infected with 5 x 10^5 trophozoites of *E. histolytica* and either 1.8 x 10^9 c.f.u./d *S. boulardii* or saline for 4 d. Rats were sacrificed on the fifth day and autopsied. In rats treated with *S. boulardii*, there were significantly less lesions than in control rats and the mean healing time of lesions was significantly less (6 d) in *S. boulardii*-treated rats compared with 21 d in the control rats.

CLINICAL USES OF *S. BOULARDII*

S. boulardii has been used in the treatment of several types of diarrhea, either as a preventive agent for antibiotic-associated diarrhea or in nasogastric tube-associated diarrhea, or as a treatment for diarrhea in adults or children associated with *C. difficile*, in chronic diarrhea in HIV-infected patients or in acute diarrhea in children and adults (Table 2). The evidence to support the efficacy for each of these conditions is described separately below.

Prevention of antibiotic-associated diarrhea

Three large studies have been completed using *S. boulardii* as a preventive therapy for antibiotic associated diarrhea (AAD). The first studied ambulatory patients in a multicentre study in France at 25 centres with a total of 388 enrolled patients [1]. Patients aged 15 years or older who received tetracycline or ß-lactam antibiotics for at least 5 d and had no intestinal pathology were enrolled in this double-blind, placebo-controlled trial. Patients were randomly assigned to *S. boulardii* (l00 mg b.i.d.) or placebo for the duration of the antibiotic. Of the 199 patients treated with *S. boulardii*, significantly fewer (nine, 4.5 per cent) developed AAD compared to patients receiving placebo (33/189, 17.5 percent, $P<0.001$).

The second double-blind study was done in hospitalised patients receiving new antibiotic prescriptions at Harborview Medical Center in Seattle, Washington [65, 67]. *S. boulardii* or placebo was assigned (2:1 randomisation, respectively) as a concurrent therapy to the antibiotics. The study drug was begun within 48 h of antibiotic initiation and continued for 2 wk after the antibiotics were discontinued. *S. boulardii* was given at a dose of 500 mg b.i.d. for a total of 1 g/d. Patients were followed for at least 8 d (range 8-57) after study initiation to observe if AAD developed and if there were any adverse reactions associated with the study drug.

Of the 180 completed patients, 14 of the 64 (21.8 per cent) on placebo developed AAD compared with 11 of the 116 (9.5 per cent) of the patients on *S. boulardii* ($\chi^2=4.31$, $P=0.038$). The efficacy of *S. boulardii* in preventing AAD was 56.7 per cent.

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Vibrio cholerae produces a toxin which activates adenylate cyclase of the enterocyte and stimulates cAMP production resulting in profound watery diarrhea. In an early study by Vidon *et al.*, [75] intestinal loops were surgically created in male Wistar rats and injected with either a 2 h pre-incubated mixture of *S. boulardii* ($3x10^9$ c.f.u./ml) and cholera toxin (10 µg/ml) or a mixture of cholera toxin and buffer (control loops). *S. boulardii* inhibited approximately 50 per cent of the fluid and sodium secretion compared with loops treated with cholera toxin alone. Irradiated or heat-killed *S. boulardii* preparations were found to have an inhibitory effect when co-administered with cholera toxin though *S. boulardii* given alone had no effect on water secretion in the jejunal loops.

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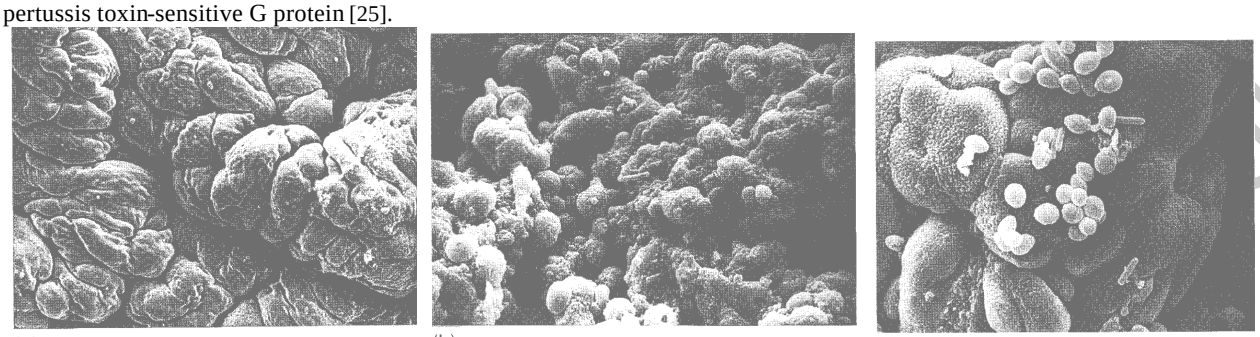

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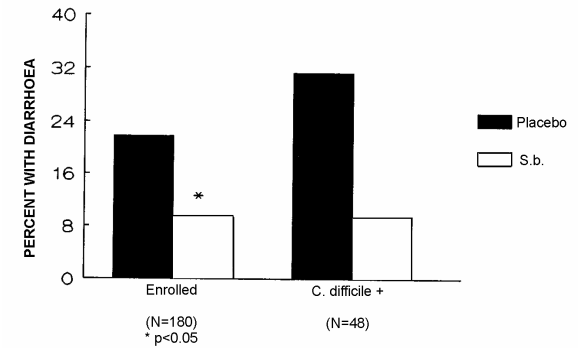

Figure 4. Incidence of antibiotic-associated diarrhea in patient populations given *S. boulardii* or placebo for an entire study population (n = 180) and for patients who acquired *C. difficile* (n = 48). *P < 0.05. (Reproduced with permission from Surawicz *et al.* 1989)

The third study tested *S. boulardii* as a preventive agent for hospitalised patients receiving at least one ß-lactam antibiotic [54]. These patients were either receiving ß-lactam antibiotics alone, or in conjunction with other antibiotics. Patients were blindly assigned (1:1) to either *S. boulardii* (500 mg b.i.d.) or placebo at least 72 h after the ß-lactam was begun and continued for at least 2 d after the antibiotic was discontinued. Patients were followed for 7wk to observe for delayed adverse reactions and the occurrence of AAD. Of the 181 eligible patients, 6/ 89 (6.7 per cent) on *S. boulardii* developed AAD which was significantly fewer than the 14/92 (15.2 per cent) of the patients on placebo who developed AAD (P=0.012). The efficacy of *S. boulardii* in preventing AAD was found to be 56 p er cent. Using multivariate analysis to control for risk factors of AAD, the relative risk of developing AAD if the patients were on *S. boulardii* was 0.26 (95 per cent confidence interval 0.08, 0.86) compared with patients on placebo. There was no adverse reactions which were significantly more common in patients receiving *S. boulardii* than in those receiving placebo. As in the previous study, in the 23 patients who were positive for *C. difficile*, the frequency of diarrhea was less in patients on *S. boulard ii* (2/10, 20 per cent) compared with patients on placebo (4/13, 31 per cent) [54].

The results from these three trials indicate that *S. boulardii* is an effective biotherapeutic agent for the prevention of antibiotic-associated diarrhea and is not associated with any significant adverse reactions in these patients.

Prevention of' nasogastric alimentation-associated diarrhea

Nasogastric alimentation may result in severe diarrhea resulting in electrolyte imbalances and in some cases sepsis [64]. Diarrhea associated with nasogastric tube alimentation may result from the impaired carbohydrate metabolism due to altered faecal flora or by the shifts of osmotic balances associated with adjusting the dose of alimentation used in each individual patient [38]. In a study of 180 hospitalised patients in Seattle, patients receiving nasogastric tube feedings had a 5.3-fold risk of developing diarrhea compared with patients not on nasogastric tube alimentation [67].

Two studies investigated the usefulness of *S. boulardii* in patients receiving nasogastic tube alimentation. The first was a double-blind randomised study in 40 patients on continuous enteral feeding admitted to an intensive care unit in France [72]. Twenty patients were randomised to *S. boulardii* (500 mg/litre of nutrient solution) and 20 patients were randomised to placebo. The duration of treatment was dependent upon length of enteral feeding (11-21 days). The patients on *S. boulardii* experienced 34 (8.7 per cent) diarrheal days during 389 days of observation and patients on placebo experienced a significantly greater number of diarrheal days (63/373, 16.9 per cent, χ^2=11.38, P<0.001). This study did not report the number of patients with and without diarrhea, so it is not possible to evaluate whether the outcome (diarrheal days) was due to a limited number of patients who had many days of diarrhea or if numerous patients contributed a few diarrheal days for the total number of diarrheal days counted.

In patients with severe burns, recovery is dependent upon an increased need for calories and nitrogen. If calorie intake is limited, reduced healing, rejection of skin grafts, and immune depression may result [77]. The effort to increase calorie intake through nasogastric tube alimentation is often frustrated by the development of diarrhea in these patients. In order to address this issue, a double-blind, placebo-controlled study was performed in patients receiving enteral alimentation on burn wards at the Cochin Hospital in Paris, France [62]. Patients in this study had major burns (20-70 per cent full-thickness burns) and were receiving nasogastric tube alimentation. They were randomly given either *S. boulardii* (2 g/d) or placebo for the duration of alimentation (from 8 to 28 d). Calorie intake was increased by 500 calories/24 h every 2 d until signs of intolerance were observed (diarrhea, vomiting, nausea, abdominal distension). The number of patients on *S. boulardii* who experienced at least one diarrheal day (2/9, 22 per cent) was less frequent than patients on placebo who had at least one diarrheal day (6/9, 66 per cent). In addition, the number of diarrheal days was significantly less if the patients were on *S. boulardii* (3/204, 1.5 per cent) compared with patients on placebo (19/208, 9.1 per cent, P<0.001). The mean calorie intake in patients treated with *S. boulardii* was significantly higher only during the second week, but was not significantly different during the entire observation period (28 d).

Although these two studies were on a limited number of patients, the results indicate that *S. boulardii* may be useful in preventing diarrhea associated with nasogastric tube alimentation. Larger studies are needed to fully evaluate the efficacy in this group of patients.

Treatment of C. difficile *diseases*

C. difficile is associated with one-third of the cases of antibiotic-associated diarrhea and nearly 99.8 per cent of all cases of pseudomembranous colitis [53]. This bacterium is associated with a spectrum of gastrointestinal disease which ranges from uncomplicated diarrhea to pseudomembranous colitis and may cause death [3,32,34]. *C. difficile* is a common nosocomial pathogen and is the most frequent known aetiology of nosocomial diarrhea [3,51]. Treatment of *C. difficile* disease ranges from discontinuation of the inciting antibiotic to active treatment with vancomycin, metronidazole or bacitracin [32]. Unfortunately, 10-20 per cent of patients treated with those antibiotics experience recurrences of *C. difficile* disease [28,32,70]. Once patients experience a recurrence of *C. difficile* disease, they are more likely to succumb to repeated recurring episodes of this disease [43]. In an effort to provide a more effective therapy which would reduce the incidence of recurrences, clinical trials were performed to test the effectiveness of *S. boulardii* as either an adjunctive therapy to antibiotic treatment against *C. difficile* or as the only treatment modality (without antibiotics) [13,30,43,66]. As discussed earlier, animal studies had indicated promise for this approach.

Kimmey *et al.* [43] published a case report of a patient with six prior episodes of recurrent *C. difficile* colitis over an 8 mth period. The patient had previously been treated with vancomycin, metronidazole, bacitracin and cholestyramine, but with no success. The patient responded to a 3 mth course of *S. boulardii* and there were no further recurrences in the 18 mth of follow-up. This observation led to an open trial of *S. boulardii* as an adjunctive treatment with vancomycin. Thirteen patients who had a history of recurrent disease were treated with 10 d of vancomycin (500 mg/d) and a 30 d course of *S. boulardii* (1 g/d). Eleven (85 per cent) successfully responded to the combination of vancomycin and *S. boulardii* and did not experience any further recurrence of disease [66].

The next study was a double-blind placebo-controlled clinical trial of 102 patients who had either recurrent episodes of *C. difficile* disease or were experiencing *C. difficile* disease for the first time [68]. Patients were given *S. boulardii* or placebo with at least four overlapping days of (vancomycin or metronidazole) treatment. The study drug was continued for 28 d (1 g/d). The patients were followed for an additional 28 d after study drug discontinuation to observe for recurrence of *C. difficile* disease. Of the 51 patients with incident (first time) *C. difficile* disease, seven of 29 (24 per cent) patients on placebo had a recurrence and two of 22 (9 per cent) patients on *S. boulardii* failed. Of the 51 patients with a history of recurrent *C. difficile* disease, 19 of 28 (68 per cent) patients on placebo had another recurrence but only nine of 23 (39 per cent) of patients on *S. boulardii* reported a recurrence. Overall, 11 of 45 (24 per cent) patients on *S. boulardii* experienced a clinical recurrence and significantly more patients on placebo had a further recurrence (26 of 57, 45 per cent, $P < 0.05$). There were no adverse reactions associated with *S. boulardii* in this study.

Buts *et al.* [11] studied 19 children aged 3 mth to 11 yr who presented with enteral symptoms lasting for more than 15 d and had *C. difficile* toxin B positive stools. The patients presented with either persisent diarrhea with malabsorption and failure to grow ($n = 8$) or repeated attacks of colic, emesis and hypermeteorism without diarrhea ($n = 4$), or both entities ($n = 7$).

All the strains of *C. difficile* tested produced *in vitro* high levels of toxin A and B. The treatment by *S. boulardii* (250 mg, two to four times per day according to age) resulted in rapid improvement of enteral symptoms (number of stools, frequency and duration of colic episodes in 18 patients, 95 per cent), and in a clearing of stool toxin B by day 15 in 16 cases (85 per cent) [11].

These studies indicate that *S. boulardii* is a safe and effective biotherapeutic agent for the treatment of gastrointestinal disease associated with a specific aetiological agent: *C. difficile.*

Treatment of acute diarrhea

Acute diarrhea in children and adults tends to be a self-limiting disease with an abrupt onset and lasting from a few days to a few weeks. The severity may depend upon the aetiology which may be due to food poisoning, intoxication, bacterial, viral or parasitic infections. In children, dehydration from the acute diarrhea may be sufficiently severe to warrant clinical concern. The challenges of clinical trials of acute diarrhea are the short duration of diarrheal symptoms and the high rate of spontaneous recovery.

Several studies have been done on the treatment of acute diarrhea in children and adults [17,18,35,39,41]. An open trial was performed in Argentina with children from 6 mth to 9 yr of age with acute diarrhea of less than 96 h duration, but not of such severity to require hospitalisation [35]. Children were given *S. boulardii* (250 mg b.i.d. for at least 4 d). Of the 22 children enrolled, 20 (91 per cent) had a resolution of diarrhea symptoms by the fourth day. Because this was an uncontrolled trial of a condition with a high rate of spontaneous cure, the effect could not be attributed to *S. boulardii*.

Chapoy tested the effectiveness of 500 mg/d of *S. boulardii* in the treatment of moderately severe acute diarrhea in infants as an adjuvant to standard oral rehydration in a randomised unblinded study [18]. Thirty-eight infants (2 wk to 30 mth) who were hospitalised for acute gastroenteritis were included. He excluded infants with weight loss greater than 10 per cent requiring intravenous rehydration, bloody or purulent stools, high fever (> 39°C) and cases of serious digestive pathology. All infants received oral rehydration. Half (n = 19) of the eligible infants were also treated with 5 d of *S. boulardii* and half of the infants (19) received only oral rehydration (controls). Of the 19 patients on *S. boulardii*, nine had identifiable stool pathogens, enteropathogenic *E. coli* (five), *Salmonella species* (four), compared with the control infants who had six with identifiable stool pathogens, enteropathogenic *E. coli* (three), *Salmonella* species (two) and *Shigella sonnei* (one), The infants on *S. boulardii* had a significant improvement of diarrhea compared with oral rehydration alone as measured by the decrease in the number of stools/day (P<0.01), decrease in the weight of stools (P<0.05), an increase in carmine red transit time (P<0.05) and an improvement of stool consistency (P<0.05). The efficiency of *S. boulardii* was found to be 80 per cent.

A similar efficacy (74 per cent) was found in a double-blind, placebo-controlled study of Mexican children with acute diarrhea by Cetina-Sauri and Basto [17]. One hundred and thirty children aged 3 mth to 3 yr were randomly assigned to either *S. boulardii* (200 mg q.i.d. for 4 d) or placebo, with both groups receiving oral electrolyte therapy. In the children receiving *S. boulardii*, 55/65 (84-6 per cent) were cured while significantly fewer children on placebo (26/65, 40 percent) were cured (χ^2 = 25.7, P<0.01).

Acute diarrhea in adults has also been studied using *S. boulardii*. Höchter *et al.* [39] tested *S. boulardii* in adult outpatients aged 18-65 with acute diarrhea. The double-blind, placebo-controlled study was conducted at eight centres in Germany. Adult outpatients were randomised to either *S. boulardii* (600 mg/d for the first 48 h, then 300 mg/d for days 3-7) or placebo. The most common identified associated cause for acute diarrhea was food poisoning, but specific aetiological agents were not determined in this study. By the eighth day, patients on *S. boulardii* did not have significantly less stool frequency compared with patients on placebo, but the proportion of liquid stools was significantly less in the *S. boulardii* group compared with patients on placebo (P = 0.03).

Treatment of chronic diarrhea in human immunodeficiency virus-infected (HIV) patients

Diarrhea in patients infected with HIV may result from a wide variety of infectious aetiologies but in a large proportion of cases, the cause remains undetermined [33]. The loss of nutrients, electrolyte imbalance and dehydration are especially dangerous in this group of immunocompromised patients. In an open trial, 17 HIV-positive patients with chronic diarrhea were given *S. boulardii* (3 g/d) for 15 d [61]. The mean number of stools per day decreased from 9.0 ± 3.2 on enrolment to 2.1 ± 0.9 on day 15 and the patients gained a mean of eight pounds in weight during the study. Thirty-five patients infected with HIV and experiencing chronic diarrhea (>24d duration) were enrolled in a double-blind, placebo-controlled trial using *S. boulardii* (1.5 g b.i.d./d for 1wk) or placebo [6]. The aetiologies of the chronic diarrhea were tested, yielding the following: *Cryptosporidium* (17 per cent), *Candida* (14 per cent), Kaposi sarcoma (8 percent), atypical *Mycobacterium* (8 per cent), CMV (8 per cent), *Mycobacterium* (6 per cent) or unknown (39 per cent). At the end of the trial, 10/18 (56 per cent) patients on *S. boulardii* were free of diarrhea *versus* 1/17 (6 per cent) patients on placebo (P<0.001).

The efficiency of *S. boulardii* in treating patients with chronic diarrhea who are HIV positive is currently being tested in a clinical trial in the USA.

SAFETY AND ADVERSE REACTIONS

The safety of *S. boulardii* has been assessed in animal models of translocation and in clinical trials in patients given *S. boulardii* or placebo. In nude mice given *S. boulardii* (5 per cent in drinking water) over a period of 70 d, there was no translocation of the yeast from the intestinal tract. Various organs (liver, kidney, lungs and heart) and mesenteric lymph nodes were harvested, minced and seeded on growth media but no *S. boulardii* were recovered [5]. In another animal model for translocation, antibiotic-decontaminated, immunosuppressed mice were challenged with either *C. albicans* alone or in combination with *S. boulardii* [4].

S. boulardii was only found in the mesenteric lymph nodes at extremely low levels (3.6 ± 1.2 c.f.u./g) compared with translocating *C. albicans* (9336 ± 2897 c.f.u./g). In addition, *S. boulardii* did not translocate at any concentration to the liver, spleen or kidney.

In two instances, *S. boulardii* has been isolated in the blood of patients receiving *S. boulardii* (1-1.5 g/d) after gastrointestinal decontamination with antibiotics. Both a child of 30 mth of age with short bowel syndrome and a history of gram-negative septicaemias [37] and an adult (33 yr old) with a colectomy and a recent history of staphylococcal and candida infections [78] were given antibiotics to decontaminate the bowel and then given *S. boulardii*. The infant developed fungaemia after 11 mth on *S. boulardii*, but the adult developed fungaemia after only 9 d on the yeast. In both cases, the fungaemia resolved after appropriate antifungal therapy. There were no further complications even after the child resumed therapy with *S. boulardii* [37].

In the clinical trials in the USA where patients were asked to document any adverse reaction noted during study drug ingestion, there were no serious adverse reactions reported more frequently in patients receiving *S. boulardii* compared with patients on placebo [54, 67, 68]. From the millions of doses of *S. boulardii* sold worldwide per year, only two cases described above of serious adverse reaction have been published. Thus, *S. boulardii* is an effective antidiarrheal biotherapeutic agent with a remarkable safety profile.

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