

## ***Saccharomyces boulardii* for *Clostridium difficile*-Associated Enteropathies in Infants**

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**Summary:** Based on experimental evidence in animals showing that the oral administration of *Saccharomyces boulardii* is effective in reducing morbidity and mortality due to *Clostridium difficile*-induced pseudomembranous colitis, we conducted an open trial to examine the effects of the living yeast, given as primary therapy, in a selected group of infants and children with persistent intestinal symptoms related to toxinogenic *C. difficile* overgrowth. Over a period of 10 consecutive months, we studied 19 eligible patients (median age 8 months) who presented with enteral symptoms lasting for > 15 days and who had solely *C. difficile* in stools with positive cytotoxin B assay. Serotyping of the strains and determination *in vitro* of production of toxins A and B were performed subsequently. The patients presented with persistent or protracted diarrhea, malabsorption, and failure to grow (n = 8), or with repeated attacks of colics, emesis, and hypermeteorism without diarrhea (n = 4), or with both entities (n = 7). Patients with chronic protracted diarrhea (n = 3) had depressed jejunal disaccharidase activities and ultrastructural changes of enterocytes, including sparse and shortened microvilli. None had evidence of colitis.

All the strains of *C. difficile* tested (n = 17) belonged to pathogenic serotypes (A<sub>1</sub>, A<sub>8</sub>, C, F, G, H, and K) and produced *in vitro* high levels of toxins A (n = 16) and B (n = 17). *S. boulardii* was given orally in a lyophilized form over 15 days (250 mg two to four times per day according to age). Within 1 week of treatment, enteral symptoms and physical findings resolved in 18 patients (95%) with marked decreases (p < 0.001) in the number of stools, frequency of colic episodes, and total duration of colics per day. Clearing of toxin B was observed within 15 days of therapy in 16 cases (85%), whereas eradication of *C. difficile* from stools was complete after 1 month in 14 (73%). Also, the ultrastructural changes observed in patients with chronic protracted diarrhea (n = 3) had disappeared after 1 month. A clinical and bacteriological relapse occurred in two patients (11%), which resolved rapidly with a second 15-day course of *S. boulardii*. These findings suggest that some toxinogenic strains of *C. difficile* may cause chronic enteropathies without colitis that may be improved by oral administration of *S. boulardii*.

**Key Words:** *Clostridium difficile* - *Saccharomyces boulardii* - Chronic diarrhea - Infancy.

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Manuscript received June 26, 1992; revision received September 1, 1992; accepted January 4, 1992.

In adults, *Clostridium difficile* is now recognized as the principal etiological agent of pseudomembranous colitis and of antibiotic-associated diarrhea (1). In neonates and infants, however, its role in intestinal diseases has been questioned (2,3). Asymptomatic carriage of nontoxigenic as well as toxigenic isolates is frequent until the age of 2 years (4), and only a few cases of pseudomembranous colitis related to *C. difficile* have been reported in infants (5,6). Although the association of toxigenic strains of *C. difficile* with chronic diarrhea, Hirschsprung's disease, necrotizing enterocolitis, and relapses of inflammatory bowel disease has been described in children (7-9), systematic antibiotic therapy for children with diarrhea and fecal *C. difficile* toxin remains controversial because of the absence of objective criteria of pathogenicity. The observation that *C. difficile* colonization is mostly the consequence of antibiotic therapy or occurs; commonly in neonates whose intestinal flora is not yet constituted emphasizes the crucial importance of the microflora as a barrier to colonization. In humans, experimental rectal infusion of homologous feces as well as the ingestion of mixed bacterial flora have been reported to be efficient in preventing relapses of intestinal symptoms related to *C. difficile* (10,11).

Moreover, the colonization of hamsters with non-toxigenic *C. difficile* strains before exposure to toxigenic strains increases survival rate up to 93% as opposed to a 21% survival rate recorded in the control group (12).

Recently, the oral administration of a lyophilized preparation of *Saccharomyces boulardii*, a non-pathogenic yeast, has also been shown to be efficient in preventing mortality due to experimental cecitis in hamsters (13) and to *C. difficile*-induced pseudomembranous colitis in gnotobiotic mice (14). A preliminary study conducted in adults has shown that the oral administration of *S. boulardii* reduced significantly the recurrence rate of postantibiotic *C. difficile* colitis (15). These observations prompted us to conduct an open trial with *S. boulardii* given orally as primary therapy in a selected group of infants and children who presented with persistent intestinal symptoms related to toxigenic *C. difficile* colonization.

## METHODS

### Patients

The study was conducted over 10 consecutive months in 19 eligible patients who all were referred to the Cliniques St-Luc, University Hospital, Brussels. Infants and children were excluded from the trial for the following reasons: clinical or endoscopic evidence of pseudomembranous colitis, immune-compromised status, antibiotic therapy for <3 days before enrollment, and discovery in stools of another pathogen. Informed consent was obtained from the parents of all infants, and the study protocol was approved by the University Hospital Ethical Committee.

Patients who were monitored for <2 weeks also were considered to have insufficient follow-up and were excluded from the study. Eligible patients had solely positive *C. difficile* stool cultures and also were positive for cytotoxin B assay. Serotyping of the strains and determination *in vitro* of toxin A and B production were performed subsequently. At enrollment, a full medical history was recorded from the parents, including details on diet, stool habits, antibiotic usage, and growth curves. *S. boulardii* (ATCC 74012 Laboratoires Biocodex, Montrouge, France) was given in a lyophilized form (batches of 250 mg) at the following doses: 250 mg two times per day for infants < 1 year of age, three times per day for children 1-4 years of age, and four times per day for those >4 years of age. Administration of the drug was continued for 2 weeks. While the infants were hospitalized, the nursing staff kept a daily record of dietary intakes, episodes of crying, colic attacks, and stool frequency and consistency. For outpatients, the same data were recorded by the parents.

The protocol called for collection of fresh stool samples for *C. difficile* culture on entry into the study and at days 7, 15, and 30. Chronic persistent diarrhea was defined as abnormally frequent (more than three per day) and liquid stools lasting for 14 days or more, and chronic protracted diarrhea as chronic diarrhea lasting for 14 days or more, associated with malabsorption, failure to thrive, and unresponsiveness to conventional therapy. Patients in whom the enteral disease resolved with treatment entered a follow-up period. The parents were asked to report whenever diarrhea or other symptoms recurred. Infants were routinely reviewed at monthly intervals. Microbiologic studies on feces were repeated at day 30 or whenever symptoms recurred. Symptomatic relapse was defined as recurrence of diarrhea and/or enteral symptoms with evidence of *C. difficile* and toxin B in the stools, provided that symptoms had resolved with initial therapy.

### Microbiological Methods

Feces were inoculated into the medium described by George et al. (16), which had been modified to include the selective agents cycloserine (350 µg/ml) and cefotaxime (4 µg/ml), and incubated at 37°C for 48 h. *C. difficile* was identified on the basis of fermentation product analysis by gas liquid chromatography. Fecal filtrates were examined for *C. difficile* cytotoxin B using confluent monolayers of HeLa cells with specific neutralization by *C. difficile* antitoxin. The serogroup of each *C. difficile* strain was determined by a slide agglutination technique using 10 rabbit antisera designated by letters A, B, C, D, F, G, H, I, K, and X (17, 18). Strains belonging to serogroup A were further characterized for subclasses (A<sub>1</sub> to A<sub>12</sub>) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (19). The strains of *C. difficile* isolated from the patients were coded and sent to one of us (G.C.) for *in vitro* analysis of toxin A and B production.

The assays were performed by persons uninformed of the identity of the strains using an enzyme-linked immunosorbent assay for toxin A and a cytotoxicity assay for toxin B (20,21).

## Statistical Analysis

Differences between means of the clinical parameters were tested for statistical significance by non-parametric methods (Mann-Whitney U test) (22).

## RESULTS

Historical data of the patients are summarized in Table 1. Of the 19 infants and children studied, 13 had received during the months or weeks preceding admission one or more courses of antibiotic therapy for acute infections, including tonsillitis, otitis, bronchitis, enteritis, or malaria. Nine infants had been treated with antibiotics for an acute episode of enteritis related to the presence of a pathogen in their stools.

Disappearance of these pathogens from stools was repeatedly demonstrated before enrollment into the study. The antibiotics incriminated for the induction of *C. difficile* infection were trimethoprim sulfamethoxazole (n = 13), amoxicillin (n = 2), erythromycin (n = 1), cloxacillin (n = 1), colimycin (n = 1), fungizone (n = 1), and chloroquine (n = 1).

**TABLE 1.** Patients and mode of presentation

Number	19
Boys	7
Girls	12
Age	
Median	8 mo
Range	2 mo to 11 yr
Patients who had received antibiotic therapies before onset	13
Patients who had acute enteritis before onset	9
Pathogens	
Rotavirus	3
<i>Y. enterocolitica</i>	3
<i>C. jejuni</i>	1
<i>S. enteritidis</i>	1
<i>C. albicans</i>	1
Mode of presentation of <i>C. difficile</i>	
overgrowth	
Chronic diarrhea	8
Colics with emesis and hypermeteorism	4
Mixed	7

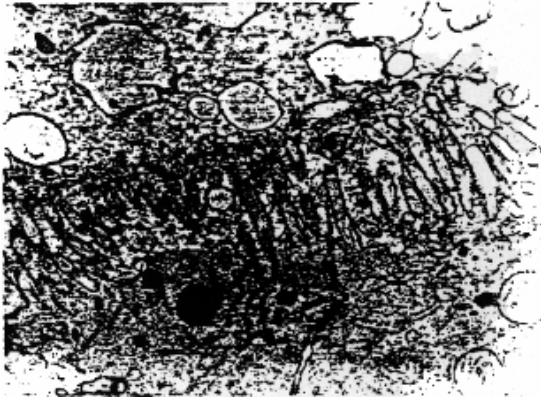
**TABLE 2.** Symptoms and clinical findings at enrollment

General		
	Weight loss, failure to thrive	6
	Poor appetite	5
	Malnutrition	3
Digestive		
	Chronic persistent diarrhea	12
	Chronic protracted diarrhea	3
	Recurrent episodes of colics	11
	Hypermeteorism	13
	Abdominal distention	8
	Repeated emesis	7

One patient had received oral vancomycin for protracted diarrhea with weight loss related to the presence of a toxinogenic strain of *C. difficile* in stools. After clinical and bacteriological relapse was confirmed, he was enrolled in the study. The patients presented on admission with persistent or protracted diarrhea (n = 8), with a syndrome of repeated attacks of colic, emesis, and hypermeteorism without diarrhea (n = 4), or with both digestive syndromes (n = 7).

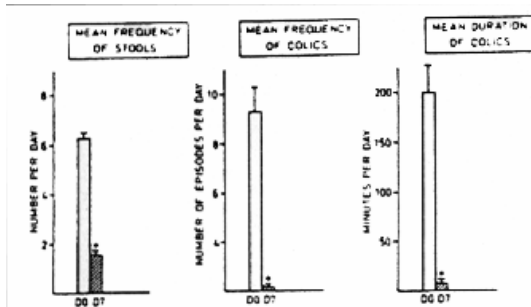
Symptoms and physical findings at admission are listed in Table 2. Among the 15 patients who presented with chronic diarrhea, three exhibited clinical and biological evidence of malnutrition with failure to grow and poor appetite. Histological examination of jejunal biopsy samples (n = 3) showed either normal mucosal morphology (n = 2) or partial villous atrophy with increased number of mucoid cells (n = 1). In two patients, the specific activity of jejunal disaccharidases was depressed to values 20-50% of the control values. Electron microscopy of jejunal enterocytes (n = 3) (Fig. 1) showed sparse and shortened microvilli covered by abundant mucoid material containing cellular debris. At rectosigmoidoscopy, however, there was no macroscopic or microscopic evidence of colitis. The patients with chronic protracted diarrhea were placed on supportive parenteral nutrition. Before therapy was initiated, all patients had *C. difficile* with positive cytotoxin B in their stools without any other pathogen detectable. Subsequent bacteriological analysis of 17 isolates from 15 patients confirmed that 16 of the 17 strains tested, produced *in vitro* high levels of both toxins A and B, one strain producing toxin B only (Table 3). Strains belonged to the following serotypes: A<sub>1</sub> (n = 2), A<sub>8</sub> (n = 1), C (n = 1), F (n = 1), G (n = 4), H (n = 5), and K (n = 3). The patients received a mean course of 15 ± 5 days of oral *S. boulardii*.

In 18 patients, symptoms and clinical findings resolved within 1 week of treatment. Compared with initial values recorded by the parents and the nursing staff on day 0, the number of stools, frequency of colic episodes, and total duration of colics per day were decreased significantly ( $p < 0.001$ ) on day 7 (Fig. 2).



**FIG. 1.** Electron microscopy of jejunal enterocytes from a 6-month-old girl with protracted diarrhea, malnutrition, and failure to thrive. A strain type H<sup>+</sup> of enterotoxigenic *C. difficile* was discovered in stools. Note shortened and sparse microvilli covered by abundant mucoid material containing cellular debris. Original magnification x29,412; reduced 50% for reproduction.

Clearing of cytotoxin B from stools occurred after 15 days in 16 patients (85%) and after 1 month in two others, whereas eradication of *C. difficile* from stools was complete after 1 month in 14 patients (73%) (Table 4). The ultrastructural changes of jejunal epithelial cells observed in patients with protracted diarrhea, including sparse and shortened microvilli, had disappeared on the control biopsy sample taken 1 month later.



**FIG. 2.** Frequency of stools, frequency of colics, and duration of colic episodes per day recorded by the parents and nursing staff on day 0 (D0) and after 7 days (D7) of treatment with *S. boulardii*. The number of patients with diarrhea is 15 and those with colic episodes is 11. Values are mean ± SD; \*p < 0.001 versus data of D0.

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**TABLE 3.** Bacteriological data: serotype of *C. difficile* and in vitro production of toxins A and B

Patients	Age	Serotype	Toxin A <sup>a</sup> (ng/ml)	Toxin B <sup>a</sup> (pg/ml)	
1. D. K	5 mo	A <sub>8</sub>	1.2	3.1	
2. D.C.	2 mo	H	1.1	3.5	
3. B.M.L.	10 mo	H	2.7	2.6	
4. K.L.	1st	4 mo	G	2.3	3.1
	2nd	6 mo	G	2.1	3.7
5. L.P.	1st	11 yr	G	2.3	4.2
	2nd	11 yr	G	2.7	3.5
6. A.C.	7 mo	K	2.3	3.6	
7. B.T.	32 mo	H	2.6	3.1	
8. W.X.	6 mo	H	3.1	3.1	
9. N.N.	9 mo	C	0.8	4.5	
10. B.S.	6 mo	A <sub>1</sub>	2.5	2.6	
11. P.S.	3 mo	K	0.5	1.3	
12. D.M.	22 mo	K	1.0	2.7	
13. D.E.	2 mo	A <sub>1</sub>	0.8	1.6	
14. C.O.	13 mo	H	0.9	3.1	
15. E.M.	4 mo	F	0.0	2.8	

<sup>a</sup> Results are expressed as log<sub>10</sub>.



Two patients (11%) in whom *C. difficile* had been eradicated were readmitted 2 months later for a clinical and bacteriological relapse consisting of the reappearance of the same symptoms and of the same toxinogenic strain (G) as found at the first episode. A second trial of 2 weeks with oral *S. boulardii* resulted in rapid disappearance of symptoms (7 days) and clearing of *C. difficile* and cytotoxin from stools (15 days). Only one patient failed to respond to *S. boulardii* therapy despite two well-conducted trials of 15 days each. This patient was admitted first at 8 months of age for protracted diarrhea, hypermeteorism, and failure to thrive lasting for 1 month. A toxinogenic strain of *C. difficile* serogroup C was discovered in the stools. After unsuccessful treatment with *S. boulardii*, oral vancomycin (40 mg/kg/day) was initiated, but the drug remained without effect on both symptoms and *C. difficile* excretion. Examination of jejunal and rectal biopsy specimens (conventional histology) showed no abnormality. Enteral symptoms and excretion of the same serogroup C strain with cytotoxin persisted in stools up to the age of 13 months, after which spontaneous disappearance of the strain and catch-up growth were observed.

**TABLE 4.** Course and outcome of the patients

No. of patients	19
Disappearance of symptoms within 1 wk	18
Disappearance of <i>C. difficile</i> from stools (total)	14
Within 15 days	9
1 mo	5
Negativation of toxin B (total)	18
Within 15 days	16
1 mo	2
Symptomatic relapses (<2 mo)	2
Reappearance of <i>C. difficile</i> and toxin B	2
Response to second trial	2
Failure of response to therapy	1

## DISCUSSION

Our study concerns a selected group of infants and children who presented with intestinal symptoms lasting for > 15 days and who all had *C. difficile* and toxin B in feces without other pathogen detectable.

Subsequent *in vitro* analysis on the fecal isolates confirmed that 17 strains tested from 15 patients all produced toxin B, whose 16 strains produced both toxins A and B. Previous studies have shown that *C. difficile* may cause chronic persistent diarrhea in infants and children (7,8). However, up to now, convincing evidence for a pathogenic role of *C. difficile* in infancy was lacking. Many asymptomatic infants colonized with *C. difficile* have detectable toxin B in feces, sometimes at titers equal to or higher than those found in adults with pseudomembranous colitis. Also, prospective studies (2,3,23) with control cases have failed to establish a clear relationship between *C. difficile* colonization, toxin production, and enteral disease.

Up to now, most of these studies have been conducted without benefit of toxin A assay because reagents for toxin A were not commercially available. Although toxin A is obviously as important as toxin B in the pathogenesis of intestinal lesions, asymptomatic neonates may be carriers of toxin A as well as of toxin B, and other pathogenic mechanisms, such as mucosal invasion, have been suggested (24). Evidence indicates that determination of the serotype of *C. difficile* is more reliable for distinguishing between pathogenic and nonpathogenic strains (18,19).

A recent study (7) has shown that some serotypes of *C. difficile* isolated from asymptomatic children, although producing toxin B, were not virulent on an animal model of pseudomembranous colitis. Using the same approach of serotype analysis, we found that all the strains isolated before therapy was initiated belonged to serotypes commonly found in adults with *C. difficile* colitis and differed from non-virulent serotypes colonizing asymptomatic neonates (4). Pathogenicity of the strains is further attested by the positive concordance between clinical and bacteriological relapses and by the persistence over 5 months in one of the patients of enteral symptoms and of a serogroup *C. strain*, usually detected in adults with severe forms of pseudomembranous colitis. Also, it is noteworthy that three patients presented with protracted diarrhea with ultrastructural changes of enterocytes consistent with a severe enteropathy unrelieved by conventional treatment, which reversed to normal within 1 month after *S. boulardii* therapy. For these patients, the average number of days of diarrhea before entry into the study was 21 days. We have observed similar cases of chronic protracted diarrhea with symptoms lasting for several months to 1 year and cured by the eradication of *C. difficile* from stools. Although the relationship between pathogenic strains of *C. difficile* and enteral lesions remains unclear, the organism has been isolated from the human jejunum and found to adhere to the jejunal mucosa, suggesting that enteral disease due to *C. difficile* has an endogenous origin (25).

The traditional approach of treating patients with *C. difficile* and related intestinal diseases is oral antibiotic therapy. Vancomycin is commonly used in adults with *C. difficile* colitis. However, although initial therapy is usually successful, relapses can occur with rates up to 50% (26,27). Because prolonged treatment with vancomycin does not clear the colon of *C. difficile* spores (27), patients who have one recurrence are even more likely to present with other recurrences. In infants and children, no clear recommendation on its use has been formulated because of the uncertainty of pathogenicity of the isolates and the cost of the therapy. Therefore, vancomycin is usually reserved for patients with confirmed pseudomembranous colitis or severe enteric disease (26). The choice of *S. boulardii* in our study was based on experimental data showing that mortality in hamsters due to clindamycin-induced cecitis was markedly decreased when the animals received the yeast (13) and on clinical evidence showing a success of this therapy in 85% of adults who had multiple recurrences of colitis after vancomycin therapy (15).

A recent prospective trial (28) has clearly demonstrated the efficacy of oral *S. boulardii* over a placebo in reducing postantibiotic-associated diarrhea in a selected group of adults at high risk for pseudomembranous colitis. Our findings in infants indicate that oral administration of the yeast resulted in prompt disappearance of symptoms (95%), with negatization of toxin B and eradication of *C. difficile* within 15 days in respectively 85% and 73% of the patients. Negatization of toxin B in stools reached 95% after 1 month. Patients with clinical and bacteriological relapses responded rapidly to a second course of *S. boulardii* with no more relapse thereafter.

The mechanism by which *S. boulardii* protects against *C. difficile* colonization and toxin production is unknown, although several properties of the yeast may contribute to explain its efficacy. In animal models of *C. difficile* overgrowth, it has been shown that the oral administration of *S. boulardii* increases intestinal resistance of the host to *C. difficile* colonization and decreases the levels of toxin B production (14-29). In a cell culture model, *S. boulardii* can prevent cytotoxicity of toxins A and B by a protective effect of the yeast on cell cytoskeleton (30). In addition, the oral administration of *S. boulardii* enhances disaccharidase activity in the intestinal mucosa of humans and rats (31), as well as the intestinal production of secretory component and sIgA (32). Although the efficacy of *S. boulardii* as primary therapy for all forms of *C. difficile* colonization awaits definitive confirmation by placebo-controlled trials, the present study suggests that this yeast may be an effective therapy in patients with persistent enteropathies related to *C. difficile*.

**Acknowledgment:** We thank Bernard Hublot, Paul Bernasconi, and François Van Hoof for their assistance and helpful comments on the manuscript; the medical nursing and technical staffs of the pediatric and microbiology department; and D. Vermeulen for typing the manuscript.

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