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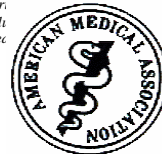
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## **A Randomized Placebo-Controlled Trial of *Saccharomyces boulardii* in Combination With Standard Antibiotics for *Clostridium difficile* Disease**

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**Objective** - To determine the safety and efficacy of a new combination treatment for patients with *Clostridium difficile*-associated disease (CDD). The treatment combines the yeast *Saccharomyces boulardii* with an antibiotic (vancomycin hydrochloride or metronidazole).

**Design** - A double-blind, randomized, placebo-controlled, parallel-group intervention study in patients with active CDD. Patients received standard antibiotics and *S. boulardii* or placebo for 4 weeks, and were followed up for an additional 4 weeks after therapy. Effectiveness was determined by comparing the recurrence of CDD in the two groups using multivariate analysis to control for other risk factors for CDD.

**Setting** - National referral study of ambulatory or hospitalized patients from three main study coordinating centers.

**Patients** - A total of 124 eligible consenting adult patients, including 64 who were enrolled with an initial episode of CDD, and 60 who had a history of at least one prior CDD episode. Patients who were immunosuppressed due to acquired immunodeficiency syndrome or cancer chemotherapy within 3 months were not eligible.

**Intervention** - Treatment with oral *S. boulardii* (1 g/d for 4 weeks) or placebo in combination with a standard antibiotic.

**Main Outcome Measure** - Recurrence of active CDD.

**Results** - A history of CDD episodes dramatically increased the likelihood of further recurrences. Multivariate analysis revealed that patients treated with *S. boulardii* and standard antibiotics had a significantly lower relative risk (RR) of CDD recurrence (RR, 0.43; 95% confidence interval, 0.20 to 0.97) compared with placebo and standard antibiotics. The efficacy of *S. boulardii* was significant (recurrence rate 34.6%, compared with 64.7% on placebo;  $P = 0.04$ ) in patients with recurrent CDD, but not in patients with initial CDD (recurrence rate 19.3% compared with 24.2% on placebo;  $P = 0.86$ ). There were no serious adverse reactions associated with *S. boulardii*.

**Conclusions** - The combination of standard antibiotics and *S. boulardii* was shown to be an effective and safe therapy for these patients with recurrent CDD; no benefit of *S. boulardii* was demonstrated for those with an initial episode of CDD.

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*Clostridium difficile* is the leading known cause of nosocomial intestinal infections and can manifest as uncomplicated diarrhea, nonspecific colitis, or pseudomembranous colitis (PMC). The incidence of *C. difficile*-associated disease (CDD) may range from 5% to 21% of hospitalized patients (1,3), and mortality may occur from complications such as toxic megacolon or intestinal perforation (24). Disease caused by this nosocomial pathogen has been shown to increase the length of stay of inpatients by a mean of 7 days, resulting in additional health care charges averaging \$10 500 per infected patient (2,5,6). The persistence of *C. difficile* as a nosocomial pathogen is facilitated by the ease of transmission within the hospital environment and by the widespread use of antibiotics known to increase the likelihood of infection (2,7). The spread of CDD is not limited to hospitals, as discharged patients colonized with *C. difficile* have been shown to cause outbreaks in extended care facilities (8,9).

Although standard antibiotic therapy with vancomycin hydrochloride or metronidazole is effective in 80% of patients with CDD, the remaining 20% of patients experience further episodes of diarrhea or colitis 3 to 28 days after the antibiotic has been discontinued (10-12). Once patients have had one recurrence, they may experience repeated episodes of disease persisting for several years (1,13,14). Patients who have only one initial episode respond well to standard antibiotics and are at low risk of having a recurrence. However, once patients fail standard antibiotics, they are at a higher risk to recur regardless of the choice of antibiotic treatment. Efforts to uncover factors that may predict why patients have recurrences and factors that can differentiate these two risk groups (initial vs recurrent disease) have not been successful (1,11,15). Other than vancomycin or metronidazole, no new antibiotic treatments have proved to be effective for CDD in large, well-controlled trials (1,16). The search for a new antibiotic treatment may not be the most effective strategy for treating CDD. *Clostridium difficile* disease occurs when antibiotics or other factors disrupt the resistance of normal colonic flora to *C. difficile* colonization, allowing overgrowth of the toxinogenic *C. difficile* to produce disease (1,17). Most antibiotics share the risk of further perturbing the protective normal flora in the colon and making the patient more susceptible to CDD recurrences (1,17). In addition, *C. difficile* has been found to carry multiple antibiotic-resistant genes (18).

The use of the yeast *Saccharomyces boulardii* for the treatment of CDD is an innovative approach, administering a microorganism as a therapeutic agent, thereby avoiding the disadvantages of antibiotics. *Saccharomyces boulardii* achieves high steady-state levels ( $10^7$  to  $10^8$  colony-forming units) in the colon within 3 days, survives gastric acids, is not absorbed, is not inhibited by antibiotics, does not significantly impact the normal flora, is cleared from the colon once therapy is discontinued, and has a good safety profile (19,22). *Saccharomyces boulardii* is effective in preventing antibiotic-associated diarrhea and in treating various forms of infectious diarrhea (23,24). This yeast is currently undergoing phase III investigational clinical trials in the United States. Evidence from animal models of CDD indicates that *S. boulardii* is effective in preventing mortality due to *C. difficile* in these models (24,25). An open trial enrolling patients with recurrent CDD found that 85% responded to a combination treatment of vancomycin and *S. boulardii* (26).

This prospective multicenter placebo-controlled trial was performed to test the hypothesis that treatment of CDD with *S. boulardii* and standard oral antibiotics (either vancomycin or metronidazole) would significantly decrease recurrences of CDD. This is the first study to analyze specific subpopulations at risk (ie, initial vs recurrent CDD) for the effectiveness of a new treatment for CDD. The rationale for the new combination treatment is twofold: first, *C. difficile* is cleared or reduced in the colon by vancomycin or metronidazole; and second, the introduction of *S. boulardii* interferes with the pathogenic processes of *C. difficile*, thereby allowing time for the reestablishment of the normal colonic flora important in the resistance to colonization by pathogenic organisms.

## METHODS

### Patient Population

The study was a national referral trial with three major study centers: University of Washington, Seattle; University of Kentucky, Lexington; and University of Michigan, Ann Arbor. Patients were recruited by referral physicians, by advertisements in local medical newsletters, and by screening *C. difficile* results at the three centers' clinical microbiology laboratories. Patients were enrolled at one of the three study centers or were referred by participating physicians from noncenter hospitals or clinics. The study was approved by the institutional review boards of each participating hospital or clinic and was conducted under an Investigational New Drug license issued by the Food and Drug Administration. Each patient gave written informed consent.

The patients were adults with active CDD ranging from uncomplicated diarrhea to PMC who were receiving one of two oral standard treatments (vancomycin or metronidazole) at the time of enrollment. In order to evaluate whether *S. boulardii* would be effective in patients with initial CDD and/or recurrent CDD, enrollment was not restricted by history of CDD. Patients were excluded from the study if any of the following conditions applied: they had chronic diarrhea due to bowel disease such as inflammatory bowel disease; were immunosuppressed by the acquired immunodeficiency syndrome (AIDS) or cancer chemotherapy within 3 months; were allergic to vancomycin and metronidazole; had negative *C. difficile* assays during the enrollment episode; were pregnant; were receiving current oral antifungal therapy; or were considered unreliable at enrollment.

### Study Drug

The study drug was taken orally at a dosage of 1 g ( $3 \times 10^{10}$  colony-forming units) per day (two 250-mg capsules, twice a day) for 4 weeks. Capsules of lyophilized yeast were prepared from actively growing broth cultures of *S. boulardii* and packaged in airtight bottles. The lyophilized yeast is stable for 3 years at room temperature. The 1:1 (*S. boulardii* to placebo) randomization and packaging of the blinded study drug kits was performed at Laboratoires Biocodex (Montrouge, France) to ensure that the study centers did not have access to the identity of the study drug. The appearance and odor of the capsules of the patented lyophilized powder of *S. boulardii* and placebo were identical.

### Study Design

The trial was conducted using a double-blind, randomized, placebo controlled, parallel-group design. A separate balanced block randomization scheme was performed for each study center (Washington, Michigan, and Kentucky). Because patients were enrolled at many sites, all patients and physicians were required to use standardized diagnostic criteria, protocols, case report forms, and *C. difficile* assay methods. At entry, a physician took the patient's medical history, obtained a stool sample for *C. difficile* assay, and started standard oral antibiotic therapy (either vancomycin or metronidazole) followed as soon as possible by the study drug (with at least 4 days of concurrent therapy). The specific antibiotic regimen was at the discretion of each patient's physician. In some cases, the type of antibiotic was switched to the other choice because of the development of hypersensitivity or lack of clinical response. The patients kept a standardized daily diary of stool frequency and consistency, other symptoms, medications, and adverse reactions. The patients were telephoned each week by a study coordinator, who verified the diary information.

At the end of study drug treatment, the patients were interviewed for adverse reactions and the daily diaries for weeks 1 through 4 were returned. The patients were followed up for an additional 4 weeks (mean time for recurrences from literature review) (1,11, 12,27) after study drug cessation, using standardized daily follow-up diaries and weekly telephone calls. At the end of the 4-week follow-up period, delayed adverse reactions were noted and the follow-up diaries were returned. Compliance with the study drug was verified by comparing the patient's medication diary with the number of capsules returned at the end of the study. Study participation was terminated by completion of study (8 weeks), study drug failure, refusal, attrition (unavailable for follow-up), or initiation of exclusion drug (such as initiating an antifungal that would be inhibitory or fungicidal for the yeast, or reinstating one of the two standard antibiotics).

### Case Definitions

Diarrhea was defined as a change in bowel habits with at least three loose or watery bowel movements per day for at least 2 consecutive days. *Clostridium difficile* diarrhea was defined as diarrhea of no other known cause (other stool pathogen, chronic gastrointestinal condition, or medications) associated with at least one positive *C. difficile* assay (culture, toxin A, or toxin B). Diarrhea cessation was defined as a return to normal bowel frequency (less than three loose or watery stools per day).

The severity of CDD was classified according to three categories: PMC, colitis or diarrhea. Pseudomembranous colitis was diagnosed by sigmoidoscopic examination revealing gross or histologically confirmed pseudomembranes. *Clostridium difficile* colitis was defined as diarrhea with fecal white blood cells or, if sigmoidoscopy was performed, evidence of focal or diffuse friability, but with no pseudomembranes. Uncomplicated diarrhea was defined as diarrhea with no documented evidence of inflammatory changes indicative of *C. difficile* colitis or PMC (1).

Patients were defined as study drug failures if they fulfilled the three following criteria: (1) 2 consecutive days of diarrhea (at least three loose stools per day); (2) a positive *C. difficile* assay or endoscopic evidence of pseudomembranes at the time of diarrhea; and (3) diarrhea symptoms were not attributable to another cause. Using these criteria, study drug failure was ascertained independently by three blinded investigators. Once the diarrhea episode was verified to be a recurrence of CDD, the patient was considered to have a study drug failure and was discharged from the study.

### *C. difficile* Culture Methods

Stool samples or rectal swabs were collected at enrollment, at the end of standard antibiotic therapy, and at the end of the 4-week blinded treatment with the study drug. The specimens were assayed for *C. difficile* by standardized culture, toxin A, or toxin B tests (1,28). Latex agglutination tests were not acceptable. All three *C. difficile* assays were performed at the three study centers in Washington, Kentucky, and Michigan on stool samples from 74 patients. The remaining 50 referral patients were enrolled by nonstudy center physicians and had stools tested for *C. difficile* by microbiology laboratories following approved, standardized culture methods or toxin B procedures (cell culture assay with neutralization) for *C. difficile* (1, 29). Toxin A was detected by commercial enzyme-linked immunosorbent assay kits (Premier Kit, Meridian Diagnostics Inc, Cincinnati, Ohio).

## Statistical Methods

The patients were evaluated on an intention-to-treat basis. All end points were included in the analysis, including standard antibiotic treatment failures, noncompliant patients, and censored patients (owing to attrition, death, or refusal). Significant differences between continuous variables were assessed by Student's *t* test. Nonparametric data were analyzed using the Mann-Whitney *U* Test. Significant differences between nominal variables were assessed by  $\chi^2$  analysis or Fisher's Exact Test. Two-tailed tests of significance were used for all tests at a  $P \leq 0.05$  level. Efficacy was calculated from the formula  $([I_p - I_T]/I_p) \times 100$ , where  $I_p$  was incidence of CDD recurrence in patients receiving placebo, and  $I_T$  was incidence of CDD recurrence in the *S. boulardii*-treated patients. Relative risks (RRs) were calculated from cumulative incidence ratios, and two-tailed 95% test-based confidence intervals (CIs) that excluded 1 were defined as significant (30). Unconditional logistic regression analysis was used to assess the relation between recurrence of CDD and treatment while simultaneously controlling for other possible risk factors of CDD. Regression variables were fitted by a nested hierarchy approach using EGRET software (Statistics & Epidemiology Research Corp, Seattle, Wash). Failure curves were calculated by the Kaplan-Meier method, with data stratified according to treatment group and history of prior CDD and compared with the Mantel Log-Rank Test. Power was calculated given the frequency of recurrences in the initial patients treated with *S. boulardii* (19.3%) or placebo (24.2%), using  $z_{\alpha} = 1.96$  and a sample size of 64.

## RESULTS

### Patient Enrollment

A total of 147 patients were initially enrolled from June 1990 through December 1992. Of those, 124 were eligible and 23 (15.6%) were not eligible for the study: 10 had a negative *C. difficile* assay at entry, six did not return enrollment forms, four were receiving exclusion drugs, one had inflammatory bowel disease, one had no current diarrhea, and one started the study drug before the antibiotic. Ineligible and eligible patients were not significantly different by mean ( $\pm$ SD) age (65.5 [ $\pm$ 16.3] years and 58.1 [ $\pm$ 20.8] years, respectively;  $t[df=145] = .75$ ;  $P > .05$ ) or site (data not shown), but significantly more who were ineligible were males (48%) compared with those who were eligible (23%) ( $\chi^2 = 4.68$ ;  $P = 0.03$ ). Patients were referred from 19 states and followed up at one of three major study centers: Washington ( $n = 50$ , 40.3%), Kentucky ( $n = 49$ , 39.5%), or Michigan ( $n = 25$ , 20.2%). The Washington center had significantly more recurrent CDD patients (80%) compared with Kentucky (26.5%) ( $\chi^2 = 26.3$ ;  $P < 0.001$ ) or Michigan centers (28%) ( $\chi^2 = 17.1$ ;  $P < 0.001$ ). There were no other differences with regard to study drug assignment, treatment efficacy, mean age, severity of CDD (diarrhea, colitis, or PMC), total days of follow-up, or other factors by study center of enrollment (data not shown). Although prescription patterns varied by the patient's history of CDD, the distribution of standard antibiotics analyzed by frequency, mean dose, or mean duration did not statistically differ by study drug group (*S. boulardii* or placebo), as shown in Table 1.

Characteristics of the study drug and placebo groups are shown in Table 2. The two groups were similar with regard to baseline characteristics, except that the patients receiving placebo had significantly more recent surgeries (Table 2). Number of medications and medical conditions were not significantly different for the two groups (Table 2). Characteristics of the enrollment episode were similar in both treatment groups (Table 3). The total dose of study drug received was also similar for the two groups (mean [ $\pm$ SD] of 23.4 [ $\pm$ 7.6] g of *S. boulardii* or 23.3 [ $\pm$ 6.8] g of placebo).

Of the 124 eligible patients, 104 (83.9%) completed the trial, nine (7.2%) were unavailable for follow-up during the study drug period (weeks 1 through 4), five (4.0%) were unavailable for follow-up during the surveillance period (weeks 5 through 8), five (4.0%) died, and one patient refused participation because of a side effect (rash due to vancomycin). Of the five patients who died during the study, one patient had been receiving *S. boulardii* (death due to pneumonia), and four patients were in the placebo group (death due to *Staphylococcus sepsis*, respiratory arrest, cardiac arrest, or prostate cancer). The mean ( $\pm$ SD) length of follow-up for patients dropped because of attrition was 20.3 ( $\pm$ 13.6) days. Attrition was not significantly different by study drug assignment or demographic variables (age or sex). From data collected in the 124 patient diaries, 14 (11.3%) were noncompliant (missed mean [ $\pm$ SD] dose of study drug of 2.0 [ $\pm$ 1.0] g). Analysis used was intent-to-treat: all 124 patients were included in the analysis.

**Table I.** Types of Combination Treatments for the Enrollment 1 by History of Prior *Clostridium difficile* Disease

Standard Antibiotic (Mean $\pm$ SD Days of Administration)	<i>Saccharomyces boulardii</i> , No. (%)	Placebo, No. (%)	<i>P</i> *	
<b>Patients With Recurrent <i>C. difficile</i> Disease (n=60)</b>				
Vancomycin (11.5 $\pm$ 3.3)	16 (61.5)	28 (82.3)	...	
Metronidazole (12.3 $\pm$ 5.5)	8 (30.8)	4 (11.8)	0.06	
Both (9.9 $\pm$ 7.6 and 8.8 $\pm$ 3.9)		2 (7.7)	2 (5.9)	0.48
<b>Total</b>	<b>26 (100)</b>	<b>34 (100)</b>		
<b>Patients With Initial <i>C. difficile</i> Disease (n=64)</b>				
Vancomycin (16.7 $\pm$ 10.2)	12 (38.7)	9 (27.3)	....	
Metronidazole (21.8 $\pm$ 12.3)	8 (25.8)	17 (51.5)	0.07	
Both (16.5 $\pm$ 9.6 and 10.5 $\pm$ 1.9)	11 (35.5)	7 (21.2)	0.53	
<b>Total</b>	<b>31 (100)</b>	<b>33 (100)</b>		

\*From Fisher's Exact Test using vancomycin as baseline.

#### CDD Recurrence

Patients given placebo reflect the expected recurrence rate in patients treated with only standard antibiotics. If patients were enrolled at their first episode of CDD, the recurrence rate was 24.2% (8/33). Patients with a history of CDD episodes were more likely to have a recurrence after standard antibiotics alone (22 [64.7%] of 34).

### Efficacy of *S. boulardii*

*Saccharomyces boulardii* was effective in patients with CDD (unadjusted RR compared with placebo, 0.47; 95% CI, 0.22 to 1.00). Using a multivariate logistic regression model to control for significant confounding factors, the RR of combination treatment failure was significantly reduced for patients treated with *S. boulardii* compared with placebo (RR, 0.43; 95% CI, 0.20 to 0.97). In the 60 patients with a history of CDD, significantly fewer patients receiving *S. boulardii* (nine [34.6%] of 26) failed combination treatment compared with those receiving placebo (22 [64.7%] of 34) ( $\chi^2=4.20$ ;  $P=0.04$ ), resulting in an efficacy of 46.5%. The 60 patients with recurrences had a mean of 3.2 prior episodes of CDD (range, one to nine) despite various treatments including vancomycin, metronidazole, bacitracin, rifampin, *Lactobacillus casei* GG, toxin binders, or miscellaneous antibiotics. The high failure rate in the placebo patients (64.7%) shows that patients with a prior CDD episode are at extremely high risk for further recurrences.

In the 64 patients with initial CDD, combination treatment failure was observed in 19.3% of patients treated with *S. boulardii* and 24.2% in patients treated with placebo; this difference was not significant ( $\chi^2=0.03$ ;  $P=0.86$ ). However, because of the small numbers of patients with initial CDD who failed, there was only a 10% power of detecting a significant difference; therefore, the result could be a type II error.

Overall, of the 124 patients enrolled in the study, 30 (44.8%) of 67 patients receiving placebo had failed treatment compared with 15 (26.3%) of 57 receiving *S. boulardii* ( $\chi^2=3.78$ ;  $P=.05$ ). The efficacy of *S. boulardii* in combination with standard oral antibiotic treatment for the prevention of CDD recurrence in the 124 patients was 41.3%. Using multivariate analysis to adjust for treatment with *S. boulardii* or placebo, the type or dose of standard antibiotic (vancomycin or metronidazole) was not associated with an increased risk of recurrence. Of all 65 patients receiving only vancomycin, 28 (43%) failed, and the mean ( $\pm$ SD) dosage was not significantly different in patients who developed recurrences than in those who did not (819 [ $\pm$ 480] mg/d and 882 [ $\pm$ 834] mg/d, respectively). Of the 37 patients receiving only metronidazole, 12 (32%) failed, and the mean ( $\pm$ SD) dosage was not significantly different in patients who developed recurrences compared with those who did not (1365 [ $\pm$ 906] mg/d and 1318 [ $\pm$ 502] mg/d, respectively). In the 22 patients receiving metronidazole and vancomycin sequentially, five (23%) failed, and the mean doses given were similar in patients who failed and patients who were cured.

Other possible risk factors for developing CDD recurrences were examined to determine if they influenced the effectiveness of the study treatment. Of the risk factors cited in the literature for CDD, only two factors were significantly associated with treatment failure in the univariate analysis: a history of CDD episodes (RR, 3.98; 95% CI, 1.80 to 8.81) and duration of CDD (RR, 1.38; 95% CI, 1.15 to 1.66). A full multivariate model that included potential confounders (age, gender, severity of enrollment episode, duration of CDD, type of antibiotic treatment, and surrogate measures for underlying medical condition) was fitted, yet only a history of CDD remained significant in the final multivariate model (adjusted RR, 3.85; 95% CI, 1.74 to 8.51). Neither the severity of the enrollment CDD episode nor the type of standard antibiotic was found to be a significant risk factor for CDD recurrence (data available from authors on request).

The severity of the recurrence episode was analyzed in the 45 patients who failed the combination treatment regimen. Patients receiving *S. boulardii* had fewer daily stools (mean [ $\pm$ SD], 2.1 [ $\pm$ 1.0]) than patients receiving placebo (mean [ $\pm$ SD], 3.3 [ $\pm$ 2.0];  $t=-2.51$ ;  $P=.02$ ) during the study drug period, but there was no difference in abdominal pain, cramps, or nausea in the two study drug groups.

The time of recurrence was similar in the two study drug groups in that most recurrences were 1 to 2 weeks after antibiotic therapy. Of the 15 CDD recurrences in patients treated with *S. boulardii*, 13 (87%) failed during the study drug period (weeks 1 through 4) and two (13%) failed during the surveillance period (weeks 5 through 8). Of the 30 recurrences in patients treated with placebo, 21 (70%) failed during the study drug period and nine (30%) failed during surveillance. Cumulative incidence of CDD recurrence is shown by the Kaplan-Meier curve (Figure).



**Table 2**-Baseline Characteristics of 124 Patients With *Clostridium difficile* Disease by Study Group

Characteristic	<i>Saccharomyces boulardii</i> (n=57)	Placebo (n=67)	Significance
Mean (±SD) age, y [range]	56.8 (±20.4)[18-95]	59.2 (±21.1)[18-94]	$t[df=122]=-0.6; P=0.52$
No. (%) female	42 (73.7)	53 (79.1)	$\chi^2=0.2; P=0.62$
History of surgery, mean No. (±SD)*	0.72 (±1.06)	1.34 (±1.9)	$t[df=107]=-2.32; P=0.02$
Inciting antibiotic, No. (%)			
Cephalosporin	16 (34.8)	17 (28.8)	$\chi^2[df=3]=1.19; P=0.75$
Penicillin	8 (17.4)	13 (22.0)	
Other single antibiotic	4 (8.7)	8 (13.6)	
Multiple antibiotics	18 (39.1)	21 (35.6)	
History of medical conditions, mean No. (±SD)	2.4 (±2.4)	2.3 (±2.09)	$t[df=122]=0.11; P=0.91$
Mean (±SD) No. of medications	31 (±2.7)	4.1 (±2.6)	$t[df=122]=-1.82; P=0.07$

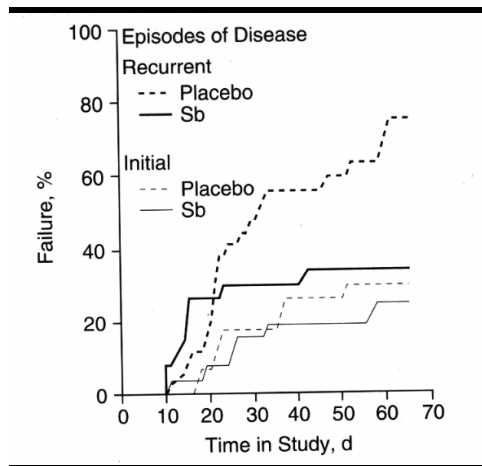
\* Surgery within 1 year of enrollment.

• Data missing from 11 patients treated with *Saccharomyces boulardii* and eight patients treated with placebo.

**Table 3**-Characteristics of Enrollment Episode of *Clostridium difficile* Disease by Study Group\*

Variable	<i>Saccharomyces boulardii</i> (n=57)	Placebo (n=67)	Significance
Initial CDD, No. (%)	31 (54.5)	33 (49.3)	$\chi^2=0.15$
Recurrent CDD, No. (%)	26 (45.6)	34 (50.7)	$P=0.70$
Median No. of prior episodes	0.0	1.0	$U=1751; P=0.39$
Severity, No.			
Diarrhea	24 (42.1)	38 (56.7)	$\chi^2[df=2]=2.68; P=0.26$
Colitis	18 (31.6)	15 (22.4)	
PMC	15 (26.3)	14 (20.9)	
Median days with CD PTE	7.0	6.0	$U=1555; P=0.07$
Median days of diarrhea PTE	10.5	11.0	$U=1749; P=0.38$
Stools per day, No. ± SD (range)	8.1 ± 6.6 (3-45)	7.3 ± 4.7 (3-23)	$t[df=101]=-0.68; P=0.50$

\* CDD indicates *C. difficile* disease; PMC, pseudomembranous colitis; PTE, prior to enrollment.



Kaplan-Meier failure curve for the probability of *Clostridium difficile* disease recurrence indicates *Saccharomyces boulardii*.

### *C. difficile* Assay

At the end of standard antibiotic treatment, 101 (81%) of the patients had a *C. difficile* assay performed, and six (12%) of the patients receiving *S. boulardii* and 10 (19%) of the patients receiving placebo were still positive for *C. difficile*. *Clostridium difficile* persistence at the end of standard antibiotic treatment did not predict an increased risk of subsequent CDD recurrence. At the end of study drug treatment (week 4 of *S. boulardii* or placebo), 76 patients (61.3%) submitted a stool specimen to test for *C. difficile*. Of note, only three (8.6%) of the 35 patients receiving *S. boulardii* continued to harbor *C. difficile* by culture or toxin compared with 11 (26.8%) patients receiving placebo (Fisher's  $P=0.04$ ). *Saccharomyces boulardii* did not significantly reduce the colonization frequency of *C. difficile* (culture positivity), but did significantly reduce the frequency of toxin B positivity (6.7%) compared with placebo (30%) ( $P=0.02$ ) by the end of week 4.

### Adverse Reactions

Two adverse reactions were reported more frequently during the study drug treatment period by patients receiving *S. boulardii* compared with patients receiving placebo. Five patients noted an increase in thirst while receiving *S. boulardii*, yet no patient on placebo reported this symptom (Fisher's  $P=0.02$ ) and eight patients reported constipation while receiving *S. boulardii* compared with two patients receiving placebo (Fisher's  $P=0.03$ ). The median time of constipation was 4 days when receiving *S. boulardii*, and seven of eight patients reported a resolution of the constipation soon after *S. boulardii* was discontinued. The frequencies of other reported adverse reactions during study drug treatment were not significantly different for the two treatment groups, and no adverse reactions for *S. boulardii* were reported during follow-up.

## COMMENT

This prospective double-blind study showed that in patients with a prior history of CDD disease, a combination treatment of *S. boulardii* and a standard oral antibiotic was more effective in preventing further CDD recurrences than standard therapy alone. Recurrence following standard antibiotic therapy is a difficult clinical problem. Diarrhea symptoms abate in most patients while receiving standard antibiotic treatment, but recurrences have been reported in 5% to 50% of patients after antibiotic cessation (1). Although it has been reported that 20% of patients have recurrences after standard therapy, the most significant risk factor found in this study (history of CDD episodes) was not evaluated in previous studies (1,12,13). These results indicate that the number of recurrences is much higher in some patients than was previously thought. History of CDD episodes profoundly increased rate of recurrence from 24% (for an initial episode) to 65% (for those with prior episodes). It has been suggested in the literature that patients who fail antibiotic treatment tend to have CDD recurrences repeatedly, and the data from this study confirmed this pattern (1,12). This pattern of repeating episodes has been reported to persist for up to 2 to 4 years, regardless of subsequent antibiotic regimes (1,11-14).

There is no uniformly effective therapy to prevent further *C. difficile* recurrences in intractable patients. Previously published therapies have included tapering or pulsed dosing of vancomycin or using alternative antibiotics such as rifampin or quinolones (1,10,13). It has been suggested that cholestyramine, a bile salt-binding resin, also binds toxin B, but this resin probably works by its nonspecific constipating effect and may also bind vancomycin (1). Another approach to treatment has been to reestablish the normal flora using biotherapeutic agents such as *L. casei* GG, rectal instillates of microbes, or fecal enemas, but large, double-blinded studies have not been reported (31-33).

Although *S. boulardii* has been found to be effective in other forms of infectious diarrhea, this is the first large double-blind study to investigate its effectiveness in patients with CDD (24). *S. boulardii* is a yeast that is found on lychee fruit, but has not been isolated as part of the normal flora. Pharmacokinetic studies indicate *S. boulardii* has a half-life of 6 hours, reaches maximum steady-state levels in 3 days, and is no longer detectable in the stool 2 to 5 days after the yeast has been discontinued (19,20, 22). Permanent colonization does not occur in humans even if the patient is exposed to antibiotics (20). While the mechanism of *S. boulardii* is not fully elucidated, a recent study reported that *S. boulardii* produces a protease that destroys the receptor site for the toxin A of *C. difficile* (34). Another recent study found that *S. boulardii* may prevent diarrhea by stimulating chloride absorption (35). In this study, *S. boulardii* was found to significantly decrease toxin B positivity frequency by week 4, but not the rate of *C. difficile* colonization (ie, culture positivity). This result supports early studies with *S. boulardii* that showed significant decreases in toxin B and toxin A positivity, but not in stool *C. difficile* concentrations (25).

Other factors that may have influenced the effectiveness of this combination approach to the treatment of recurrent CDD were analyzed. The effectiveness of *S. boulardii* was not influenced by the choice of standard antibiotic (vancomycin or metronidazole) or by the dose per day given to the patient. The effectiveness of *S. boulardii* was similar in patients with uncomplicated diarrhea (47.3%), colitis (37.5%), or PMC (46.6%). Previous studies of CDD have reported several risk factors for nosocomially acquired CDD (advancing age, female sex, type of inciting antibiotic, and chemotherapy) (3-5,36), but few studies have elucidated factors that may predict repeated recurrences of CDD (11,15,16). Unlike the risk factor profile of nosocomial CDD, the only risk factor for further recurrences found in this patient population was history of CDD. After adjusting for this risk factor using multivariate analysis, *S. boulardii* continued to be protective. In this study, patients with initial CDD responded well to the standard antibiotic; but the recurrence rate was too low to determine whether a significant, difference existed between the treatment groups.

The limitations of the trial were largely intrinsic to the community-based referral design and current clinical practices. Because of the nature of the disease, patients were referred to the trial by physicians across the United States. Thus, by the time of enrollment, the choice of antibiotic regimen was predetermined by the patient's primary physician. Diagnoses are not routinely made by biopsy; thus, some of the patients may have had more severe disease than was documented in the enrollment forms. The documentation of the clinical progress was dependent on standardized patient diaries, but study coordinators confirmed these data by weekly telephone calls. Instability of viable *S. boulardii* in stool samples and problems of transport limited the ability to perform consistent stool cultures for *S. boulardii* during and after treatment. Surveillance was done for 4 weeks after study drug discontinuation, which may have missed some patients with delayed recurrences but, as observed in the placebo-treated patients, 70% recurred within 3 weeks. This is consistent with other reported recurrence times (1,16).

Patients who were immunosuppressed due to acquired immunodeficiency syndrome or cancer chemotherapy were not eligible for the study; safety and efficacy of *S. boulardii* in these patients has not been determined.

The combination treatment of a standard antibiotic (either vancomycin or metronidazole) with *S. boulardii* appeared to be a safe and improved therapy for these patients with recurrent CDD and may represent a more effective approach than standard antibiotic treatment alone.

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