

## Prevention of $\beta$ -Lactam-Associated Diarrhea by *Saccharomyces boulardii* Compared with Placebo

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**Objectives:** To determine the safety and efficacy of a new preventive agent for antibiotic-associated diarrhea (AAD) in patients receiving at least one  $\beta$ -lactam antibiotic. **Methods:** A double-blinded, placebo-controlled, parallel group study was performed in a high-risk group of hospitalized patients receiving a new prescription for a  $\beta$ -lactam antibiotic and having no acute diarrhea on enrollment. Lyophilized *Saccharomyces boulardii* or placebo (1 g/day) was given within 72 h of the start of the antibiotic(s) and continued until 3 days after the antibiotic was discontinued, after which the patients were followed for 7 wk. **Results:** Of the 193 eligible patients, significantly fewer, 7/97 (7.2%), patients receiving *S. boulardii* developed AAD compared with 14/96 (14.6%) on placebo ( $p = 0.02$ ). The efficacy of *S. boulardii* for the prevention of AAD was 51 %. Using a multivariate model to adjust for two independent risk factors for AAD (age and days of cephalosporin use), the adjusted relative risk was significantly protective for *S. boulardii* (RR = 0.29, 95% CI = 0.08, 0.98). **Conclusion:** The prophylactic use of *S. boulardii* given with a  $\beta$ -lactam antibiotic resulted in a significant reduction of AAD with no serious adverse reactions.

## INTRODUCTION

Although many newly developed antibiotics have broad spectrums of activity and fewer side effects, antibiotic-associated diarrhea (AAD) remains a common complication. The incidence of AAD has ranged from 3.2 to 29/100 in studies of hospitalized patients (1-3). The onset of AAD may be rapid (while the patient is on antibiotics) or may be delayed for up to 6 wk after the antibiotics have been discontinued (4, 5). The severity of AAD may range from uncomplicated to severe diarrhea and serious complications may arise, including electrolyte imbalances, dehydration, pseudomembranous colitis, toxic megacolon, or death (1, 2, 6). The occurrence of AAD in hospitalized patients has also been associated with increased length-of-stays by 8-20 days, higher medical care costs, five-fold increased risks of developing other nosocomial infections, and three-fold increases in mortality (3, 68). Identified etiologies of AAD include *Clostridium difficile* (25-30%), and to a lesser extent, *Salmonella*, *C. albicans*, and enterotoxigenic *Clostridium perfringens*; but the etiologies of the majority of AAD remain unclear (1, 4, 9). Speculation on the causes of AAD include: 1) the overgrowth of *C. difficile*; 2) the unveiling of toxin receptors or attachment sites caused by the disappearance of the normal flora; 3) the decrease in short-chain fatty acids due to the loss of bacterial strains responsible for the metabolism of complex carbohydrates; or 4) the lack of nutrient competition caused by changes in normal flora (10-14). The various theories of the etiology of AAD are tied together by the common impact of antibiotics on the normal colonic flora. Previous studies have found that neither the dose nor duration of antibiotic increases the risk of AAD, but the type of antibiotic may be important (1, 3, 15). Antibiotics with a spectrum of activity that includes anaerobic bacteria (especially cephalosporins, penicillins, or clindamycin) have been associated with higher rates of AAD, although nearly all types of antibiotics have been implicated (1, 16-21). The most frequently implicated broad spectrum antibiotics are those agents that impact the anaerobic component of the fecal flora; consequently,  $\beta$ -lactam antibiotics have been found to have the highest frequencies of AAD (1, 2, 16, 22).

Treatment modalities for AAD are limited because no established treatment exists for non-*C. difficile*-associated AAD except for discontinuing the inciting antibiotic and supportive care (1). Treatment for *C. difficile*-associated AAD may require oral metronidazole or vancomycin, but 20% of the patients may develop subsequent recurrences after antibiotic treatment ceases (4).

*Saccharomyces boulardii* is a nonpathogenic yeast that has been used in Europe as an anti-diarrheal agent (23, 24). Results from *in vivo* studies have shown that *S. boulardii* reaches high, steady state levels in the Stool ( $10^7$ - $10^8$ ) within 3-5 days and is no longer detectable by 2-6 days after discontinuation of the yeast (25-27). *S. boulardii* has been shown to be effective in the treatment of *C. difficile* colitis and, in one study by Surawicz *et al*, *S. boulardii* was effective in preventing AAD in patients with a wide variety of types of antibiotic exposure (28-30). Because of the frequent use of  $\beta$ -lactam antibiotics and the high risk of AAD associated with these types of antibiotics, we performed a double-blinded, placebo-controlled trial of *S. boulardii* in patients receiving at least one type of  $\beta$ -lactam for the prevention of AAD.

## MATERIALS AND METHODS

### *Patient population*

Consecutive adult inpatients receiving new prescriptions for at least one  $\beta$ -lactam antibiotic were screened at one of four hospitals: University of Washington Medical Center and Harborview Medical Center, Seattle, Washington; University of Kentucky Medical Center, Lexington, Kentucky and St. Louis University Medical Center, St. Louis, Missouri. The Human Subjects Review Committee at each center approved the study protocol, and each patient gave written informed consent. All patients were adult (18-86 yr) inpatients who received a new prescription of a  $\beta$ -lactam antibiotic, either alone or with another antibiotic, for at least 48 h and had no diarrhea less than 24 h after enrollment. In the case of different antibiotics given sequentially, patients were eligible if the antibiotics were started less than 7 days before enrollment, and the time between antibiotic courses (one being a  $\beta$ -lactam) was less than 48 h.  $\beta$ -lactam antibiotics included medium-to-broad spectrum penicillins, combination penicillins (penicillins with a  $\beta$ -lactamase inhibitor), or any cephalosporin. Patients receiving only penicillin G or penicillin V (narrow spectrum penicillins) were not eligible for the trial.

Patients were assigned to either oral *S. boulardii* or placebo at 1 g ( $3 \times 10^{10}$  colony-forming units) per day (2 250-mg capsules, twice a day). The study drug assignment was randomized within three age groups for each center (aged 18-44, 45-69, or 70-99). The appearance and odor of the capsules of the patented *S. boulardii* and placebo were identical. The 1:1  $\phi$ . *boulardii*:placebo randomization and packaging of the blinded study kits was performed at Laboratoires Biocodex (Montrouge, France) to ensure that the study investigators did not have access to the identity of the study drug. The study drug was started within 72 h of the  $\beta$ -lactam antibiotic and continued for 3 days after the antibiotic was discontinued. The maximum duration of study drug treatment was 28 days. After the discontinuation of the study drug, the patients were followed for a total of 7 wk, which is 1 wk longer than the mean incubation period for AAD quoted in the literature (4, 5). During follow-up, data was collected on clinical symptoms and delayed adverse reactions, which included physical symptoms, fever, rash, changes in blood chemistries, urinary indicators (protein, BUN, glucose), or changes in liver enzymes. The patients were given a standardized daily diary to record stool frequency and consistency, antibiotics, other medications taken, and any adverse reactions. The patients were also followed by study investigators daily while hospitalized and were phoned weekly after discharge.

### Case definitions

An eligible antibiotic was an antibiotic given for at least 48 h by an oral or intravenous route. Multiple antibiotics were defined as antibiotics given either simultaneously with the  $\beta$ -lactam antibiotic or sequentially (with a maximum of 48 h between the last dose of the first antibiotic and the start of the second antibiotic).

Diarrhea was defined as a change in bowel habit with at least 3 loose stools/day for at least 2 consecutive days. AAD was defined as diarrhea associated with at least one  $\beta$ -lactam antibiotic with no other etiology of diarrhea identified (medications, lactose intolerance, nasogastric tube feedings, enemas). The etiology of all cases of diarrhea was determined independently by three blinded investigators. Study drug failure was defined as a patient developing AAD either while on the antibiotic or at any time during the 7 wk of follow-up after the study drug was discontinued. Study termination was either by completion of the study or by censoring because of: refusal, death, initiation of a new antibiotic while no longer on study drug, attrition, or the initiation of an exclusion drug (oral antifungal).

A modified standard index (APACHE) was used to quantitate the patient's basic health status to stratify acutely ill patients (31). The modified APACHE index substituted oral for rectal temperature, SGOT, SGPT, total protein, and serum glucose for respiratory data, arterial pH, serum sodium, and serum potassium. This APACHE index was verified on a separate data base, in which 144 patients had complete blood chemistries, and was found to have a mean score of  $3.9 \pm 2.5$  for patients with mild underlying disease and a significantly higher score (mean =  $5.9 \pm 2.9$ ,  $t = 4.5$ ,  $p < 0.001$ ) for patients with more severe underlying disease conditions (28).

### Antibiotic prescription patterns

To determine the trends of antibiotic use at two of the study hospitals (Harborview Medical Center and University of Washington Medical Center), antibiotic purchase inventories were collected from the pharmacies, and the total units used were calculated from cost per unit and total annual cost for each type of antibiotic from 1989-1992 where data was available.

### Microbiological methods

Stool samples or rectal swabs were collected on enrollment, at the end of study drug treatment, and at any time that diarrhea occurred. Inoculum from stool or rectal swabs was plated onto Difficile agar plates (Prepared Media Laboratories, Tualatin, Oregon) or CCFA (BBC Laboratories) and incubated anaerobically for 48 h at 37°C. To facilitate detection of *C. difficile* at low levels, an aliquot of stool was also inoculated into prereduced supplemented peptone broth (Becton Dickinson Vacutainer System, Rutherford, NJ), containing 39  $\mu\text{g/ml}$  cefoxitin and 0.1% pure sodium taurocholate, and incubated for 72 h at 37°C. *C. difficile* was identified using standard procedures (32). Stools were also tested for cytotoxin within 48 h of collection using CHO cell tissue cultures. Diluted stool (1:1) was centrifuged (3000 RPM for 10 min) and filtered (0.8  $\mu\text{l}$  pore size). Serially diluted filtered stool was then added to cell cultures and observed for cytopathic effect at 24 h. The specificity of the cytopathic effect was checked by neutralization using *Clostridium sordellii* anti-toxin (33).

### Statistical methods

The patients were evaluated on an intention-to-treat basis to provide a more valid assessment of treatment efficacy in routine clinical practice (34). All patients were included in the trial including completed and censored patients (owing to attrition, death, refusal, or initiation of exclusion drugs). Differences between means were assessed using the Student's  $t$  test, differences between group proportions were assessed using the  $\chi^2$  statistic or, if the sample size was small, Fisher's exact test. Nonparametric data was analyzed using the Wilcoxon ranked sum test. To test the hypothesis that the incidence of AAD was decreased in the patients receiving *S. boulardii* compared with the incidence of AAD in patients receiving placebo, a binomial exact test was used to determine a  $p$  value (35). Two-tailed tests were used to test the significance at a  $p \leq 0.05$  level for factors that were not known *a priori* to increase or decrease the incidence of AAD. The efficacy was determined using the equation:  $[(I_p - I_i)/I_p] \times 100$ , where  $I_p$  is the incidence of AAD in the patients receiving placebo and  $I_i$  is the incidence of AAD in patients receiving *S. boulardii*. Unadjusted relative risks were calculated from the formula  $(1/I_p)$ , and 95% confidence intervals were calculated (36). Failure curves were calculated by the Kaplan-Meier method, with data stratified according to treatment group and compared with the Mantel log-rank test. Logistic regression analysis was used to assess the relation between AAD and treatment while simultaneously controlling for other possible risk factors of AAD. Regression variables were fitted by a nested hierarchy approach using EGRET software (Statistics & Epidemiology Research Corporation, Seattle, Washington). Coefficients of the regression variables were tested for significance using differences of log likelihood statistics interpreted as  $\chi^2$ .

## RESULTS

### Enrollment

During the study enrollment period (March 1989-December 1992), 12,546 patients were screened for entry. Reasons for noninclusion included: antibiotic started  $>72$  h of interview (24%), immunosuppression (15%), antibiotic given  $<48$  h (10%), catastrophic illness (9%), no telephone (7%), discharged before interview (6%),  $<18$  yr old (4%), on oral anti-fungal medication (4%), refused participation (2%), and miscellaneous (19%). Of the 208 patients enrolled in the study, 15 were ineligible for the following reasons: study drug was initiated  $>72$  h after the antibiotic(s) were begun ( $n = 9$ ), oral anti-fungal drug started  $<48$  h from enrollment ( $n = 2$ ), diarrhea  $<24$  h after enrollment ( $n = 1$ ), and antibiotic or study drug given for  $<48$  h ( $n = 3$ ). To determine if selection bias may have occurred, a comparison of the ineligible patients with the eligible patients was performed; there were no significant differences by age, gender, APACHE score, number of antibiotics or medications on enrollment, reasons for antibiotic prescription, or assignment to *S. boulardii* or placebo (data not shown).

Of the 193 eligible patients, 129 (67%) completed the trial, 25 (13%) were censored during the study drug period, and 39 (20%) were censored during the 7-wk follow-up period. Of the 64 censored patients, 28 were lost to follow-up, 27 received a new antibiotic prescription poststudy drug, four developed adverse reactions (nausea or constipation), three died, and two received oral nystatin. A comparison of the 129 completed patients with the 64 censored patients revealed no significant differences by study drug assignment, gender, age, antibiotic use, enrollment site, or APACHE score (data not shown). Censored patients had significantly shorter mean follow-up times than completed patients ( $19 \pm 2$  vs  $55 \pm 20$  days, respectively,  $t = 14.7$ ,  $p < 0.001$ ), received less total g of study drug ( $12.4 \pm 8.1$  vs  $16.0 \pm 7.5$  g, respectively,  $t = 3.1$ ,  $p < 0.01$ ), and developed less AAD (4.7% vs 14.0%, respectively, Fisher's  $p = 0.04$ ), but none of these factors were significantly different by the type of study drug received (*S. boulardii* or placebo) (data not shown).

#### *Treatment groups*

Of the 193 eligible patients, 97 were assigned to *S. boulardii* and 96 were assigned to placebo. To judge if bias was introduced by treatment assignment, a comparison of the group of patients receiving *S. boulardii* and the group receiving placebo was performed. No statistically significant differences were noted in the patients assigned to *S. boulardii* compared with patients given placebo (Table 1). Patients treated with *S. boulardii* received a mean of  $2.4 \pm 1.0$  antibiotics, which was similar to the mean number of antibiotics ( $2.5 \pm 1.1$ ) received by patients on placebo. The types of antibiotics and medications were not significantly different in patients on *S. boulardii* or placebo (data not shown).

#### *Site comparison*

Because patients were enrolled at four hospitals, a comparison of the patients by enrollment site was performed (Table 2). Patients from the four sites were generally similar except for differences in age, number of medications, and APACHE index. Patients at St. Louis University were significantly older, had higher APACHE index scores, and received more antibiotics and medications; but none of these factors resulted in a higher frequency of AAD. Patients at the University of Kentucky had a significantly lower frequency of AAD, were older, had higher APACHE scores, and had significantly lower frequency of *C. difficile* (Table 2).

**TABLE 1**  
Baseline Characteristics by Study Drug Group

	<i>Saccharomyces boulardii</i> (n = 97)	Placebo (n = 96)	p value
Age (Mean ± SD)	40.7 ± 16.0	42.3 ± 17.7	t = -0.65 p = 0.51
Randomized age groups			
18-44	64 (66.0%)	62 (64.6%)	c <sup>2</sup> = 0.27
45-69	25 (25.8%)	24 (25.0%)	df = 2
≥ 70	8 (8.2%)	10 (10.4%)	p = 0.87
Gender			
Male	62 (63.9%)	63 (65.6%)	c <sup>2</sup> = 0.009
Female	35	33	df = 1
			p = 0.92
History of antibiotics* (Mean ± SD)	0.43 ± 0.68	0.35 ± 0.60	t = 0.86 p = 0.39
History recent surgery	16 (16.7%)	23 (24.0%)	c <sup>2</sup> = 1.16 p = 0.28
APACHE (Mean ± SD)	7.96 ± 4.11	7.97 ± 4.68	t = -0.03 p = 0.97
Number of medications (Mean ± SD)	6.9 ± 5.0	7.8 ± 5.8	t = -1.17 p = 0.24
Number of antibiotics (Mean ± SD)	2.4 ± 1.0	2.5 ± 1.1	t = -0.65 p = 0.52

\* Any antibiotics given 6 wk before eligibility antibiotics.

**TABLE 2**  
Comparison of Study Variables by Enrollment Site

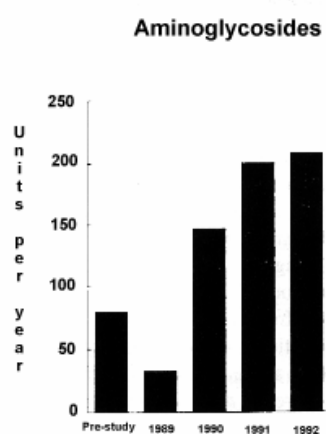
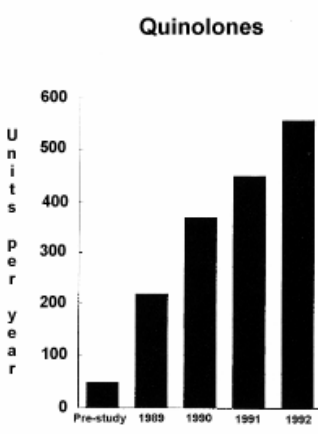
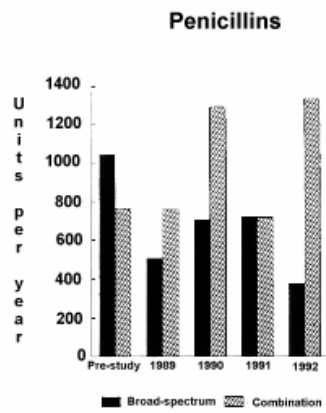
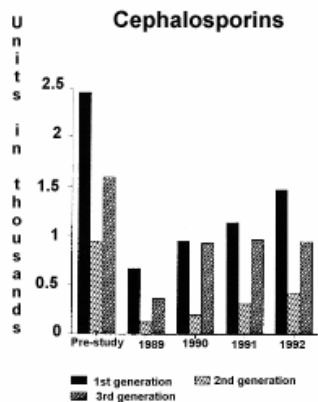
	HMC (n = 79)	UH (n = 13)	St. Louis (n = 39)	Kentucky (n = 62)
Study drug				
<i>S. boulardii</i>	41 (51.9%)	6 (46.2%)	19 (48.7%)	31 (50%)
Placebo	38 (48.1%)	7 (53.8%)	20 (51.3%)	31 (50%)
Outcome				
AAD	12 (15.2%)	3 (23.1%)	3 (7.7%)	3 (4.8%)*
No AAD	67	10	36	59
Age (Mean ± SD)	38.2 ± 14.4	32.5 ± 14.8	47.8 ± 18.5*	43.7 ± 17.7*
APACHE (Mean ± SD)	6.6 ± 3.3	6.5 ± 3.4	10.4 ± 5.0*	8.3 ± 4.6*
N antibiotics (Mean ± SD)	2.2 ± 1.0	2.1 ± 1.1	2.8 ± 0.8*	2.5 ± 1.1
N medications (Mean ± SD)	6.9 ± 4.3	5.5 ± 5.2	9.4 ± 5.0*	7.0 ± 6.2
<i>C. difficile</i>				
Positive	19 (24%)	5 (38%)	0	0*
Negative	60	8	2	39
Follow-up (Mean days ± SD)	53.6 ± 27.5	49.9 ± 23.0	48.2 ± 26.7	45.7 ± 24.2

\*  $p < 0.05$  compared with HMC as base line.

HMC, Harborview Medical Center; UH, University Hospital; St. Louis, St. Louis University Medical Center; Kentucky, University of Kentucky Medical Center.

### Frequency of AAD

Of the 193 eligible patients, 21 (10.9%) experienced AAD during the study period. Seven patients reported diarrhea that did not meet the case definition of AAD. Blinded assessment assigned the cause of nonantibiotic-associated diarrhea to: nasogastric tube feeding ( $n = 3$ ), laxative use ( $n = 2$ ), lactose intolerance ( $n = 1$ ), and viral gastroenteritis ( $n = 1$ ). The mean incubation period of AAD (from the first day of the antibiotic to the first day of diarrhea) was 18 days and ranged from 3 to 56 days. The severity of AAD was measured by the duration of AAD (which ranged from 2-25 days) and stool frequency (which ranged from 3 to 9 loose or watery stools/day) and the presence of fever (oral  $\geq 101^\circ\text{F}$ ) in 9.5% of the patients with AAD. In patients on placebo, the duration of AAD was shorter (median of 4 days) while patients were on antibiotics (which may have reflected the rapid response to discontinuation of the inciting antibiotic), but the duration was prolonged (median of 18 days) for patients with delayed AAD (when discontinuation of the antibiotic is no longer a treatment option). In patients on placebo, the frequency of AAD was higher for patients on multiple antibiotics (15%) than patients on a single  $\beta$ -lactam (11.8%).



**FIG. 1.** Antibiotic-use patterns over time. Data was compiled from two study hospitals (Harborview Medical Center and University of Washington Medical Center, Seattle, Washington). Pre study refers to data collected from the same data source from 1986 to 1989. Combination penicillins include penicillins with  $\beta$ -lactamase inhibitors.

### Antibiotic-use trends

Basic antibiotic-use trends were recorded at selected study hospitals during the period of patient enrollment to observe if time trends of antibiotic use were associated with the changing frequency of observed AAD (Fig. 1). Use of first- and second-generation cephalosporins gradually increased, and third-generation cephalosporin use remained constant during the last 3 yr of the study. Broad-spectrum penicillin use decreased from 1988 to 1992, whereas the use of combination penicillins (those with  $\beta$ -lactamase inhibitors) increased. The use of narrow-spectrum antibiotics (aminoglycosides) and other broad-spectrum antibiotics (quinolones) increased over the study years. The total number of prescribed antibiotic units generally remained stable from 1989 to 1993. The frequency of AAD in the total study population remained fairly constant: in 1989 (11.3%), 1990 (11.2%), 1991 (13.3%), and 1992 (8.7%), even though there were major changes in the types of antibiotics used by these hospitals over the same time period.

**TABLE 3**  
Factors Associated with Antibiotic-Associated Diarrhea

Factor	Patients with AAD (n = 21)	Patients without AAD (n = 172)	Total (N = 193)	p value*
Study drug				
<i>S. boulardii</i>	7 (7.2%)	90	97	$p = 0.03^{**}$
Placebo	14 (14.6%)	82	96	
<i>C. difficile</i>				
Positive	7 (29%)	17	24	$\chi^2 = 4.59$ $p = 0.03$
Negative	11 (10%)	98	109	
Net determined	[3]	[57]		
Age blocks***				
18-44	18 (14.3%)	108	126	$\chi^2 = 5.46$ $p = 0.06$
45-69	1 (2.0%)	48	49	
>69	2 (11.1%)	16	18	
Cephalosporin duration (Mean days $\pm$ SD)	10.3 $\pm$ 12.9	6.8 $\pm$ 7.4	—	$t = 1.77$ $p = 0.08$
Antibiotic exposure				
Single $\beta$ -lactam	4 (11.4%)	31	35	Fisher's $p = 0.55$
Multiple	17 (10.8%)	141	158	

\*  $p$  values from unadjusted estimators unless otherwise noted.

\*\*  $p$  value from binomial probability.

\*\*\* Age within *a priori* randomized blocks.

### Risk factors for AAD

In unadjusted estimates of increased risk for AAD, two factors were found to be significantly associated with AAD (Table 3): *C. difficile* positivity and assignment to placebo. Two other factors showed a trend ( $0.05 < p < 0.08$ ) of increasing the risk for AAD (age < 45 yr and increasing days of cephalosporin use). Stool *C. difficile* cytotoxin or culture assays were available from 133/193 (69%) of the eligible patients. In the 133 patients tested for *C. difficile*, 7/18 of the cases of AAD were associated with *C. difficile*. Of the 24 patients with positive *C. difficile* assay results, seven had AAD and 17 were asymptomatic carriers of *C. difficile*. The incidence of AAD was significantly greater in *C. difficile*-positive patients (7/24, 29.2%) than in *C. difficile*-negative patients (11/109, 10.1%  $\chi^2 = 4.59, p = 0.03$ ). The unadjusted relative risk for *C. difficile* was 2.9 (95% CI = 1.25, 6.69). The mean age of patients with or without AAD was not significantly different ( $37 \pm 15$  yr and  $42 \pm 17$  yr, respectively), but there was a trend of patients with AAD being younger (18-44 yr old,  $p = 0.06$ ). Because of the study design, all patients received high-risk  $\beta$ -lactam antibiotics, and the comparison between penicillins and cephalosporins did not yield significant differences of AAD development. Patients with AAD did have a trend for longer exposures to cephalosporins (Table 3). Medical history variables (history of gastrointestinal surgery, smoking, alcohol abuse, allergies, or prior hospitalizations) were also similar in patients with AAD and without AAD (data not shown). Enrollment characteristics (gender, APACHE, type of primary infections, chronic conditions) and types of medications received during the study were not significantly associated with AAD (data not shown).

### Efficacy of *Saccharomyces boulardii* for AAD

Seven (7.2%) of the 97 patients receiving *S. boulardii* developed AAD compared with a significantly higher frequency (14/96, 14.6%) of patients assigned to placebo ( $p = 0.02$ ). Thus, the efficacy for *S. boulardii* in preventing AAD was 51%. The unadjusted relative risk of developing AAD for patients on *S. boulardii* compared with patients on placebo was 0.48 (95% CI = 0.23, 0.97). The characteristics of AAD in the two study groups is shown in Table 4. Of the seven patients who received *S. boulardii* and developed AAD, five developed AAD while on antibiotics, and the remaining two patients developed AAD during the follow-up period. Of the 14 patients on placebo, eight developed AAD while on antibiotics, and the other six had delayed AAD. Although the diarrhea severity (as measured by daily stool frequency) was not significantly different for the two groups, the duration of diarrhea was significantly less for patients on *S. boulardii* both while on antibiotics and postantibiotic AAD (Table 4). *S. boulardii* also appeared to slow the development of AAD, as reflected by longer incubation periods observed in the *S. boulardii*-treated patient group (Table 4).

The efficacy of *S. boulardii* was examined by the type and number of antibiotics received by the patients. There was no significant difference for AAD in the two study groups given single  $\beta$ -lactams, but there was a trend for a lower frequency of AAD in patients on *S. boulardii* compared with placebo receiving multiple antibiotics (6% vs 15%, respectively,  $p = 0.06$ ). Of the 97 patients given a penicillin and at least one other antibiotic, the frequency of AAD was significantly lower (2.4%) in the 42 patients on *S. boulardii* compared with the 55 patients on placebo (16.4%, Fisher's  $p = 0.02$ ). Of the 116 patients given a cephalosporin and at least one other antibiotic, the frequency of AAD was 6.9% in the 58 patients on *S. boulardii* and 15.5% in the 58 patients on placebo, but this difference was not significant (Fisher's  $p = 0.12$ ).

**TABLE 4**  
*Characteristics of Antibiotic-Associated Diarrhea by Study Drug Group*

Characteristic	<i>S. boulardii</i> (n = 7)	Placebo (n = 14)	p value
Number with AAD			
During antibiotic/study drug	5	8	Fisher's
Posttreatment (7-wk follow-up)	2	6	$p = 0.44$
Total	7	14	
Incubation period (Mean days $\pm$ SD)			
During antibiotic/study drug	11.2 $\pm$ 6.5	7.1 $\pm$ 7.2	$t = 4.18$ $p < 0.01$
Posttreatment (7-wk follow-up)	28.5 $\pm$ 12.0	20.7 $\pm$ 14.9	$t = 4.04$ $p < 0.01$
Duration of AAD (median day)			
During antibiotic/study drug	3.0	4.0	$t = 26.0$ $p < 0.05$
Posttreatment (7-wk follow-up)	2.5	18.0	$t = 24.5$ $p < 0.05$
Diarrhea severity (Mean stools/day $\pm$ SD)			
Total	4.9 $\pm$ 2.2	5.2 $\pm$ 1.2	$t = 1.2$ , NS

NS, not significant.

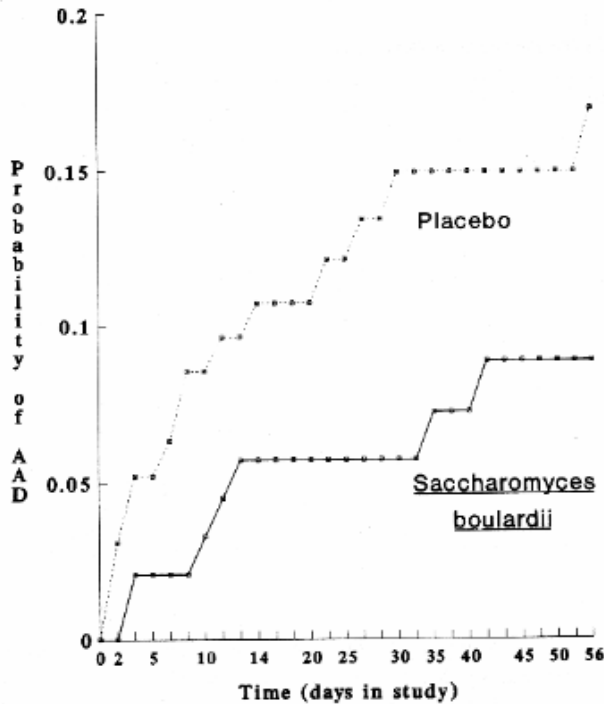
In the 24 patients with positive *C. difficile* assays, the frequency of AAD was not significantly different by the type of study drug assignment; 3/10 of the patients on *S. boulardii* developed AAD compared with 4/14 on placebo. The power of detecting a significant difference based on the sample size of 24 and the above rates was less than 3%.

A multivariate unconditional logistic regression model was used to adjust for risk factors and for *a priori* randomized age blocks to obtain a risk estimate for the study drug independent of these factors. Two factors were found to be significant in the multivariate model: age and increasing days of cephalosporin use. The types, dose, duration, or number of other antibiotics were not significant after the two risk factors had been included in the model. The multivariate adjusted relative risk for the study drug showed significant protection against AAD by *S. boulardii* (RR = 0.29, 95% CI = 0.08, 0.98). The final model with the above factors was significantly predictive of AAD ( $\chi^2 = 11.3$ ,  $p = 0.02$ ). As shown on the Kaplan-Meier Curve (Fig. 2), by the end of the study significantly more patients receiving placebo developed AAD compared with patients receiving *S. boulardii* ( $\chi^2 = 3.71$ ,  $p = 0.05$ ).

To assess if there was a bias due to censoring of patients, the effectiveness of *S. boulardii* was compared in completed and censored patients. The multivariate adjusted relative risk of developing AAD for patients receiving *S. boulardii* remained significantly protective (RR = 0.33, 95% CI = 0.02, 0.67) when other factors were adjusted for (length of follow-up, dose of study drug, and censoring status).

*Safety and adverse reactions*

Of the 193 enrolled patients, 185 (96%) returned completed adverse reaction forms. There were no significant adverse reactions with the exception that placebo patients reported more intestinal gas ( $n = 7, 7.4\%$ ) than *S. boulardii*-treated patients ( $n = 0, p = 0.01$ ) and significantly more patients given placebo reported fever ( $n = 5, 5.3\%$ ) compared with *S. boulardii*-treated patients ( $n = 0, p = 0.04$ ).



**FIG. 2.** Kaplan-Meier probability curve for the probability of developing AAD. The patients on *S. boulardii* ( $n = 97$ ) are denoted by the *solid line*, and patients on placebo ( $n = 96$ ) are denoted by the *dotted line*.

## DISCUSSION

Even with the advent of newer improved antibiotics, AAD remains a clinical concern. This trial focused on a high-risk group of patients, namely those receiving  $\beta$ -lactam antibiotics, to test a new prophylactic agent for AAD, and *S. boulardii* was found to be effective. Previous trials with AAD have involved the treatment of acute AAD, but a prophylactic agent would be most beneficial because this would decrease the medical complications and cost of treating acute disease. The severity of AAD in this study was significant as judged by the duration of diarrhea (median 4.5 days, range 2-25 days), frequency (mean 5 stools/day), and presence of fever (9.5%). None of the patients with AAD had endoscopic examinations, so it is unknown if colitis or pseudomembranous colitis was present. However, in patients with *C. difficile* disease, the diarrhea was sufficiently severe in 57% of the patients to require treatment with vancomycin or metronidazole.

The frequency of AAD is dependent on several factors and may be high in some populations. The incidence in this study was not trivial (14.6 cases of AAD/100 enrolled patients on placebo). The rate of AAD at one of the study hospitals (U. of Kentucky) was lower despite an older, sicker study population, and this may have been due to the lower prevalence of *C. difficile*, the most common infectious etiology of AAD. The frequency of AAD in a specific institution may also vary over time because of factors such as changes in antibiotic prescribing patterns, patient demographics, or in-hospital infection control procedures (2, 3, 17). The incidence of AAD in patients (given placebo) at one institution (Harborview Medical Center) fell from 22% in a study conducted from 1986 to 1989 (19) to 16% in this study done from 1989 to 1992. This decrease in AAD was noted despite the observation that patients in the more recent study were sicker (mean APACHE of 8) compared with the patients in the earlier trial (mean APACHE of 5). Third-generation cephalosporin and broad-spectrum penicillin use decreased during this study (Fig. 1) whereas the use of aminoglycosides and quinolones increased. In other studies, increases in AAD incidence have also been associated with increases in third-generation cephalosporin use (17, 37, 38). Our hypothesis for the observed decreasing rate of AAD was the decrease in third-generation cephalosporins used during this study period.

In this study, *S. boulardii* was found to decrease the frequency of AAD by 51%. Of interest, *S. boulardii* not only decreased the number of patients developing AAD, but also significantly shortened the duration of AAD once it occurred. The most dramatic impact was in patients with delayed AAD when the duration of disease fell from a median of 18 days in placebo-treated patients to 2.5 days in *S. boulardii*-treated patients. Animal studies have revealed that short term (5 days) treatment with common antibiotics (such as penicillin G, ampicillin, tetracycline, or cefuroxime) had long term effects (2-10 wk) on the normal flora (39-41). In this study, 8/21 cases occurred postantibiotic exposure with incubation periods ranging from 3 to 46 days postantibiotics, indicating that the human colon may also require a significant time period for the normal flora to recover its protective effect. Another effect of *S. boulardii* was to slow the development of AAD, as reflected by the longer incubation periods in the *S. boulardii*-treated group, although the clinical relevance of this finding is unclear. Pharmacokinetic studies have shown that *S. boulardii* is cleared quickly (3-5 days) from the colon after its discontinuation, thus the residual effects of *S. boulardii* found in this study may be due to the normalization of colonic flora facilitated during *S. boulardii* administration and not to a direct action of *S. boulardii* (24-27). In addition, the effectiveness of *S. boulardii* was more pronounced in patients who received multiple antibiotics, which is a group at higher risk for AAD.

The mechanisms of action of *S. boulardii* have been explored by several researchers. Pothoulakis *et al* found that viable *S. boulardii* produces a protease that interferes with *C. difficile* toxin A binding to specific intestinal receptors (42). In addition, *S. boulardii* exerts trophic effects on the intestinal mucosa, resulting in an increase in secretory component, secretory IgA, and in intestinal enzymes such as lactase, maltase, and sucrase (43, 44). Pharmacokinetic studies have also shown elevated stool steady-state levels of *S. boulardii* in antibiotic-treated rat and human volunteers compared with nonantibiotic-exposed controls (26, 27). Thus, in the antibiotic-disturbed intestine at high risk for development of AAD, the yeast is present at high levels ( $>10^8$  CFU/g stool).

Other factors that may have influenced the effectiveness of *S. boulardii* were analyzed. The analysis of the relative risks for AAD by specific antibiotic types was limited in this study because of the requirement that all patients be on at least one high-risk  $\beta$ -lactam antibiotic. The high frequency of study patients receiving multiple antibiotics reflected current clinical prescription patterns, and this clinical practice further increases the risk of AAD to patients. Several studies have shown that the risk of AAD increases not only with  $\beta$ -lactam antibiotics, but also with the use of multiple antibiotic exposure (1, 16, 22, 28). Other reported risk factors for AAD besides antibiotics have included: age, enteral feeding, recent enemas, use of antacids/H<sub>2</sub> blockers, presence of *C. difficile*, longer lengths of stays in hospitals, and more severe underlying disease conditions (17, 21, 22). *C. difficile* has been reported to be associated with 20-40% of the cases of AAD (1, 3, 19). *C. difficile* was found to be a significant risk factor for AAD in this study (unadjusted relative risk = 2.9) and was cultured in 39% of the patients with AAD. The low number of patients who acquired *C. difficile* limited the multivariate analysis of this factor and also limited the evaluation of the effectiveness of *S. boulardii* in *C. difficile*-positive patients. There was only a 3% power to detect a difference if there was one, thus this may be due to a type II error. In a previous study with more *C. difficile*-positive patients ( $n = 48$ ), a trend for a lower frequency of *C. difficile*-associated AAD was found in *S. boulardii*-treated patients (3/32, 9.4%) compared with placebo patients (5/16, 31%,  $p = 0.07$ ) (28). In trials that were specifically aimed at the treatment of acute *C. difficile* disease, *S. boulardii* was found to be a significantly effective treatment, especially for patients with recurrent *C. difficile* disease (30, 45, 46). The risk factors found in other studies (length of stay, surgery, enemas, antacid use) were not found to be significant in this patient population.

*S. boulardii* was shown in this study to be a safe biotherapeutic agent that significantly reduced the incidence of  $\beta$ -lactam-associated diarrhea, either given alone or with other antibiotics. *S. boulardii* may offer a safe and effective means of preventing AAD in patients in whom diarrhea would be highly undesirable.

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