

# Dose–Response Efficacy of a Proprietary Probiotic Formula of *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R for Antibiotic-Associated Diarrhea and *Clostridium difficile*-Associated Diarrhea Prophylaxis in Adult Patients

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**OBJECTIVES:** Standard therapies for antibiotic-associated diarrhea (AAD) and *Clostridium difficile*-associated diarrhea (CDAD) have limited efficacy. Probiotic prophylaxis is a promising alternative for reduction of AAD and CDAD incidence.

**METHODS:** In this single-center, randomized, double-blind, placebo-controlled dose-ranging study, we randomized 255 adult inpatients to one of three groups: two probiotic capsules per day (Pro-2,  $n=86$ ), one probiotic capsule and one placebo capsule per day (Pro-1,  $n=85$ ), or two placebo capsules per day ( $n=84$ ). Each probiotic capsule contained 50 billion c.f.u. of live organisms (*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei* LBC80R® Bio-K+ CL1285). Probiotic prophylaxis began within 36 h of initial antibiotic administration, continued for 5 days after the last antibiotic dose, and patients were followed for an additional 21 days.

**RESULTS:** Pro-2 (15.5%) had a lower AAD incidence vs. Pro-1 (28.2%). Each probiotic group had a lower AAD incidence vs. placebo (44.1%). In patients who acquired AAD, Pro-2 (2.8 days) and Pro-1 (4.1 days) had shorter symptom duration vs. placebo (6.4 days). Similarly, Pro-2 (1.2%) had a lower CDAD incidence vs. Pro-1 (9.4%). Each treatment group had a lower CDAD incidence vs. placebo (23.8%). Gastrointestinal symptoms were less common in the treatment groups vs. placebo and in Pro-2 vs. Pro-1.

**CONCLUSIONS:** The proprietary probiotic blend used in this study was well tolerated and effective for reducing risk of AAD and, in particular, CDAD in hospitalized patients on antibiotics. A dose-ranging effect was shown with 100 billion c.f.u., yielding superior outcomes and fewer gastrointestinal events compared to 50 billion c.f.u. (ClinicalTrials.gov number NCT00958308).

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## INTRODUCTION

Antibiotic-associated diarrhea (AAD) is a common complication that occurs in 5–25% of adult patients, depending on the type of antibiotic that is administered (1). Although mild cases are often treated with conservative measures, AAD may progress in severity to result in colitis, dehydration, electrolyte disturbance, bowel perforation, and megacolon. A common cause of AAD is infection with the bacterium *Clostridium difficile*. In fact, most AAD cases that result in colitis are caused by *C. difficile* (2). This condition, commonly referred to as *C. difficile*-associated

diarrhea (CDAD), has progressively increased in incidence and severity over the last decade, largely due to the common use of broad-spectrum antibiotics (3). CDAD is a major public health concern and accounts for significant morbidity and mortality, extended hospitalization, and greater health-care expenses, especially in patients with recurring episodes and in the elderly (4–7).

Current standard prevention treatments for AAD and CDAD have limitations (8). Mild cases are typically treated with discontinuation of the offending antibiotic and with dietary changes. However, severe cases often require bed rest, intravenous fluids, and additional

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antibiotics such as metronidazole or vancomycin. Unfortunately, almost one in four patients treated with antibiotics for CDAD will relapse within 2 months and over half of patients with two or more previous episodes will relapse (7). Furthermore, significant morbidity and expense are associated with continued antibiotic use. There is an obvious need for new AAD and CDAD prevention treatments that are safer and more effective than the current options.

Probiotics are a promising therapy that may prevent AAD and CDAD as well as reduce mortality, morbidity, and health-care costs (9–11). Probiotic bacteria enhance the host flora by stimulating immune function and suppressing pathogenic bacteria colonization (12). Several meta-analyses have concluded that various strains of probiotics can decrease AAD and CDAD incidence in adults (13–15). However, many of the trials on this topic are plagued by methodological flaws such as lack of power, limited sample size, and lack of dose-ranging outcomes.

The current randomized, double-blind, controlled study represents the largest trial of probiotic therapy for AAD and CDAD prevention to date. Furthermore, this is the first trial to examine dose-ranging outcomes with this promising therapy. We tested two hypotheses in this trial. First, would probiotic prophylaxis lower the incidence of AAD and CDAD in hospitalized adults receiving antibiotic therapy? Second, would this effect occur in a dose-dependent manner?

## METHODS

This single center, three-arm, randomized, double-blind, placebo-controlled dose-ranging study was conducted at the Xinhua/Yuyao Hospital (Shanghai, China), which is affiliated with Shanghai Jiao Tong University School of Medicine (Shanghai, China). All research procedures performed in this trial were in strict accordance with a predefined protocol that was approved by all researchers and the local ethics committee. The ethics committee approved the study protocol on 8 October 2008 and participants gave informed consent before participation. This study is registered under ClinicalTrials.gov number NCT00958308. The CONSORT statement for randomized trials served as a template for reporting this clinical study (16).

### Participants

Eligible patients were hospitalized for various types of infections and received antibiotic therapy with penicillin, cephalosporin, or clindamycin. Inclusion criteria were age 50 to 70 years, hospitalization of 5 or more days, and antibiotic therapy of at least 3 days but no more than 14 days. Exclusion criteria were use of other probiotic products, active diarrhea, noncontrolled intestinal disease, documented *C. difficile* infection within the 3 months before enrollment, immunosuppressive therapy, antibiotic use within 30 days of enrollment, or active participation in another clinical study.

### Interventions

Study products were made available, formulated, and produced by Bio-K+ International (Laval, Quebec, Canada). The product was stored at 4°C in a secure area until dispensed and administered by the assigned clinical staff. Patients were randomized into one

of the three study groups: two probiotic capsules per day (Pro-2,  $n=86$ ), one probiotic capsule and one placebo capsule per day (Pro-1,  $n=85$ ), or two placebo capsules per day ( $n=84$ ). Each commercially available probiotic capsule contained 50 billion c.f.u. (*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei* LBC80R®).

Patients received the initial dose of the assigned intervention within 36 h of their prescribed antibiotic therapy, and continued daily usage of the product for 5 additional days after completion of their antibiotics. Patients were then followed for an additional 21 days after completion of the assigned intervention. Patients took their daily dose 2 h after breakfast and antibiotic administration each day.

### Outcomes

Standard baseline assessments included a complete physical examination, medical history, blood pressure, and body mass index. If diarrhea occurred while hospitalized, patients provided a stool sample for *C. difficile* analysis of Toxin A and/or B. All episodes were recorded by a nurse or designated clinician on a case report form using the seven-item Bristol Stool Form Scale (17). A diarrhea episode was defined as a bowel movement consisting of watery stool with or without solids. Diagnosis of AAD was made when a patient produced three or more liquid stools in a 24-h period after antibiotic treatment with no other obvious reason for diarrhea. Duration of diarrhea was determined by number of continuous days of diarrhea. Average number of liquid stools per day was determined by the sum of the number of liquid stools per day in the AAD episode divided by the duration of diarrhea in days.

The stool from patients presenting with two or more liquid stools was tested for *C. difficile* by a triage panel and the cytotoxin cell culture assay, performed on all specimens tested by triage, which were antigen positive and Toxin A negative. Furthermore, all stools were tested for *C. difficile* for the second diarrhea episode and considered *C. difficile* positive when it was antigen and Toxin A and/or B positive.

Episodes of AAD and gastrointestinal disorders during hospitalization were recorded by patient interview and were confirmed by review of patient diaries. Posthospitalization episodes were recorded in patient diaries and confirmed by patient interview.

### Sample size

The sample size of this trial ( $n=255$ , 85 per group) provided a minimum statistical power of 86% to detect a significant dose-response relationship and to allow for a maximum 12% dropout rate in each group.

### Randomization

The randomization sequence used in this trial was generated by a computerized random-number generator (SAS, release 9.2; SAS Institute, Cary, NC) using a permuted block design that randomized among the three study groups while stratifying for age (50–59 vs. 60–70 years) and number of days on antibiotics (3–8 and 9–14 days). Study products were delivered to the investigative site in identical containers labeled only with the lot number and a sequentially numbered patient identification code.

### Masking

This study was conducted using triple-blinding procedures. First, patients were blinded to the treatment received throughout the trial. Each patient received two pills each day, which were identical in shape, size, taste, smell, and color regardless of the assigned treatment group. Second, investigators and all involved clinicians were blinded to the treatment allocation throughout the course of the study. Finally, all study coordinators, clinical monitors, and biostatisticians were blinded to treatment allocation throughout the entire clinical study and until after all analyses were completed. This methodology is the most effective and stringent design to minimize study bias.

### Statistical methods

All data were recorded on case report forms, double-entered, verified, and independently monitored for accuracy by Sprim Advanced Life Sciences (San Francisco, CA). All analyses were performed according to the intention-to-treat principle, i.e., outcome measures were based on the original denominator of 255 patients and each patient analyzed according to original treatment assignment. Continuous variables, e.g. days with AAD, are reported as mean±s.d. Categorical variables, e.g. incidence of AAD, are presented as *n* (%). Further group comparisons were assessed with  $\chi^2$ -test or Fisher's exact test. No adjustments were made for multiplicity. Statistical analyses were performed using SAS/STAT software (release 9.2; SAS Institute).

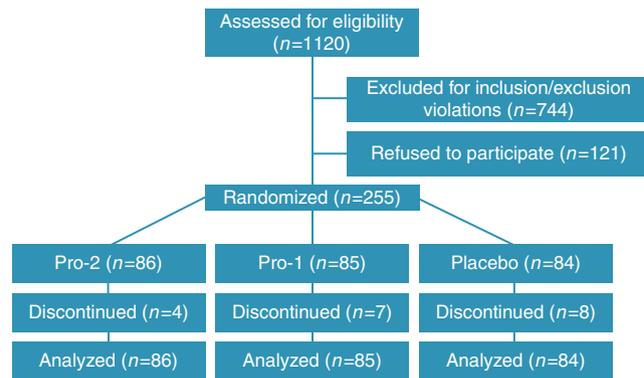
## RESULTS

Of the 1120 patients who were eligible to participate in the study, 865 were excluded from participation (**Figure 1**). The remaining 255 patients were enrolled in the trial between January 2009 and March 2009. Nineteen (7.5%) patients did not complete the study (Pro-2, *n*=4; Pro-1, *n*=7; placebo, *n*=8). All failures to complete the study were due to personal reasons and not related to the study. These patients still returned for regular follow-up visits and none experienced an episode of AAD or CDAD. There was full concordance with the study treatment by the remaining 236 patients. All patients were of Asian ethnicity. No major differences were observed in baseline characteristics among the three study groups (**Table 1**).

A distinct dose–response relationship was observed as higher probiotic dosage resulted in a lower incidence of AAD (**Figure 2**). Furthermore, both probiotic dosages were more effective in reducing AAD vs. the placebo group. Similar relative benefits were observed across the three treatment groups regardless of age and antibiotic time course strata (**Table 2**).

Patients treated with probiotics reported fewer ( $P<0.001$ ) days with continuous AAD vs. the placebo group (**Table 3**). The duration of AAD symptoms in the Pro-2 group was 32% shorter compared to the Pro-1 group. Similar relative improvements were observed across age and antibiotic time course strata.

A dose–response relationship was also observed regarding the incidence of CDAD, which lowered with higher probiotic dosages (**Figure 3**). In the placebo group, 23.8% (20/84) of patients



**Figure 1.** CONSORT patient flow diagram. Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

**Table 1.** Baseline patient characteristics

Characteristic	Group		
	Placebo (n=84)	Pro-1 (n=85)	Pro-2 (n=86)
Age (years)	60±6	60±6	60±6
Sex (no. (%))			
Male	42 (50)	43 (51)	46 (54)
Female	42 (50)	42 (49)	40 (46)
Days on antibiotic (mean±s.d.)	8.4±3.2	8.4±3.3	8.2±3.6
Comorbidities (no. (%))			
COPD	23 (27)	18 (21)	14 (16)
Chronic bronchitis	12 (14)	13 (15)	14 (16)
Hypertension	5 (6)	14 (17)	10 (12)
Diabetes mellitus	6 (7)	8 (9)	12 (14)
Heart disease	6 (7)	12 (14)	7 (8)
Tracheitis	6 (7)	6 (7)	8 (9)
Digestive ulcer	1 (1)	4 (5)	1 (1)
Pneumonia	0	1 (1)	2 (2)
Gastritis	0	0	1 (1)
Type of administered antibiotic (no. (%))			
Cephalosporins	35 (42)	35 (41)	28 (33)
Penicillins	26 (31)	19 (22)	30 (35)
Lyncomycin	23 (27)	31 (36)	28 (33)

COPD, chronic obstructive pulmonary disease.  
All *P* values were >0.05.

were positive for CDAD. In comparison, only 9.4% (8/85) of Pro-1 patients ( $P=0.03$  vs. placebo) and 1.2% (1/86) of Pro-2 patients ( $P=0.002$  vs. placebo) were positive for CDAD. Furthermore, fewer Pro-2 patients ( $P=0.04$ ) were CDAD positive vs. Pro-1 patients. Similar relative benefits were observed across the three treatment groups regardless of age and antibiotic time course strata (**Table 2**).

The incidence of abdominal pain, abdominal distension, loose stool, and constipation during the trial was lower ( $P < 0.05$ ) with higher dosages of probiotic therapy. Specifically, the incidences in the placebo, Pro-1, and Pro-2 groups were 40.5%, 24.7%, and 12.8% for abdominal pain; 35.7%, 21.2%, and 9.3% for abdominal distension; 58.3%, 44.7%, and 31.4% for loose stool; and 14.3%, 11.8%, and 8.1% for constipation.

Three (1.2%) patients reported a nonserious adverse event during the study. Two adverse events occurred in the placebo group (fever and hematochezia) and one occurred in the Pro-2 group (fever). None of the adverse events were deemed study related.

## DISCUSSION

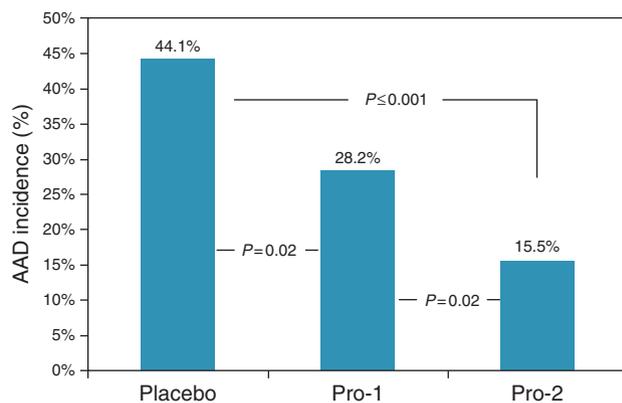
This randomized, double-blind, controlled dose-ranging study showed a significantly lower incidence of AAD and, in particular, CDAD for both probiotic treatment groups compared to the placebo group. Furthermore, a distinct dose-response effect was observed with higher probiotic dosages resulting in greater efficacy, shorter time with continuous AAD, and fewer gastrointestinal symptoms.

The results of this study compare favorably with similar trials. Plummer *et al.* (18) reported that patients taking a probiotic containing *Lactobacillus* and *Bifidobacterium* had a 2.9% CDAD

incidence vs. 7.3% in a placebo group. Hickson *et al.* (19) studied 135 patients randomized to either probiotics (*L. casei*, *L. bulgaricus*, and *Streptococcus thermophilus* formula) or placebo. Patients treated with probiotics had a lower incidence of AAD (12% vs. 34%) and CDAD (0% vs. 17%) and reported no side effects. This study included patients who were prescribed with cephalosporin, penicillin, and clindamycin, the so-called high-risk antibiotics, which were excluded in the Hickson study. Therefore, this study shows the efficacy of the *Lactobacilli* formula in the prevention of CDAD even when the most offending antibiotics are used. Furthermore, neither the Plummer nor the Hickson trial reported outcomes with multiple probiotic dosages.

Although meta-analytic techniques have suggested that higher probiotic dosages may be associated with better outcomes (20), this is the first clinical study to show this finding. In light of the dose-response relationship shown in this study, we propose that positive patient outcomes may be partly due to the high probiotic dosage (50–100 billion c.f.u. per day) compared to other similar trials (mean, 3 billion c.f.u. per day) (14). Although the mechanism of action has not been fully elucidated, the probiotic load of this quantity likely overwhelms the intestinal tract and repopulates the gut with nonpathogenic flora, as well as enhances immune response to inhibit or destroy pathogenic bacteria (20).

Preventative probiotic therapy may be especially appealing for older patients and for patients on prolonged antibiotic administration. For example, 62% of patients in the placebo group, in the 60–70 year stratum, and the 9–14 days antibiotic length stratum acquired AAD. In comparison, Pro-2 patients in the same strata had only a 24% AAD incidence. Relative risk reductions of 62–68% were observed with Pro-2 vs. placebo across all strata. Given that 61 AAD cases occur per 100,000 population with greater incidence in the elderly (21), we found that probiotic administration has potential to prevent approximately 4 million AAD cases worldwide each year. It has also been suggested that probiotic administration may reduce hospital costs associated with CDAD by 50% vs. standard therapies (18). With a 23% lower absolute risk of CDAD with Pro-2, the number needed to treat to prevent one case of CDAD is only five patients. Overall, probiotic therapy is a particularly appealing therapy due to its efficacy, safety, and potential for cost savings. In contrast, the current standard of care for CDAD—antibiotic treatment with metronidazole or vancomycin—has distinct limitations.



**Figure 2.** Antibiotic-associated diarrhea incidence by study group. AAD, antibiotic-associated diarrhea; Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

**Table 2.** AAD and CDAD incidence by age and antibiotic time course

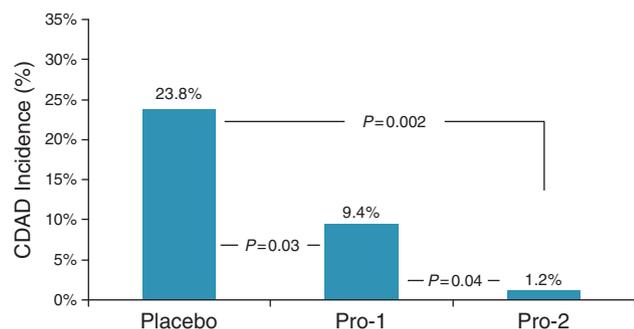
Age (years); antibiotic time course (days)	Group					
	Placebo (n=84)		Pro-1 (n=85)		Pro-2 (n=86)	
	AAD incidence (%)	CDAD incidence (%)	AAD incidence (%)	CDAD incidence (%)	AAD incidence (%)	CDAD incidence (%)
50–59; 3–8	28.6	19.1	23.8	9.5	9.1	0
50–59; 9–14	42.9	23.8	27.3	9.1	14.3	0
60–70; 3–8	42.9	23.8	28.6	9.5	13.6	0
60–70; 9–14	61.9	28.6	33.3	9.5	23.8	4.8

AAD, antibiotic-associated diarrhea; CDAD, *Clostridium difficile*-associated diarrhea; Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

**Table 3.** Duration of AAD symptoms by age and antibiotic time course

Age (years); antibiotic time course (days)	Group		
	Placebo (n=37/84)	Pro-1 (n=24/85)	Pro-2 (n=13/86)
Total patients	6.4±1.8	4.1±1.5*	2.8±0.8*†
50–59; 3–8	4.3±0.8	2.2±0.8	1.5±0.7
50–59; 9–14	5.2±0.7	3.7±0.5	2.7±0.6
60–70; 3–8	6.1±1.1	4.5±1.1	2.7±0.6
60–70; 9–14	8.2±1.0	5.6±1.0	3.4±0.6

Mean ±s.d. calculated in patients diagnosed with antibiotic-associated diarrhea. Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics. \* $P < 0.001$  vs. placebo; † $P = 0.04$  vs. Pro-1.



**Figure 3.** *Clostridium difficile*-associated diarrhea incidence by study group. CDAD, *C. difficile*-associated diarrhea; Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

Metronidazole is inexpensive but has marginal efficacy whereas vancomycin is more effective but is very expensive (7,22). Furthermore, AAD and CDAD often recur after treatment with either of these therapies and their continued use compounds the growing worldwide problem of antibiotic resistance (23,24). Overall, there is growing evidence to support the use of probiotics over antibiotics for prevention of AAD and CDAD.

This study had several limitations worth mention. First, the present results are specific to the product studied and cannot be generalized to other probiotic products (25). Second, although AAD and CDAD often begin between 4 and 9 days after antibiotic use is stopped, they can occur up to 8 weeks later. Therefore, it is plausible that some late cases of AAD and CDAD were missed. Third, we enrolled only patients aged 50–70 years in this study, which may limit applicability to younger patients. However, we feel that this is a minor limitation because AAD/CDAD is most common in older patients and because probiotics have been successfully used for these conditions in patients of all ages (14). Fourth, the length of antibiotic therapy in this study was between 3 and 14 days. Therefore, the effects of probiotic administration on AAD/CDAD with prolonged antibiotic treatment are unknown. Finally, patients in this study were solely of Asian descent. Therefore, caution must be exercised when applying these study outcomes to patients of other ethnic origins.

The evidence for probiotic prophylaxis in AAD and CDAD has been hindered by trials with inconsistent study outcomes, especially in adult patients, small sample sizes, and poor study quality (13–15,20,26). This clinical study differs from previous trials of probiotic therapy on AAD and CDAD in several ways. First, this study is the largest randomized controlled trial of probiotic therapy for AAD and CDAD to date. Second, this study is the first to report greater efficacy with higher probiotic dose. Finally, this trial used very stringent data collection and data analysis methods including triple blinding, double data entry with verification, independent data monitoring, and an intent-to-treat analysis. Overall, we believe that this study represents the highest quality trial of probiotic prophylaxis for AAD and CDAD in adults.

The proprietary probiotic blend of *Lactobacillus acidophilus* CL1285® + *Lactobacillus casei* LBC80R® (Bio-K + CL1285) studied in this clinical trial was effective in reducing risk of AAD and, in particular, CDAD and was well tolerated in hospitalized adult patients on antibiotic therapy. A dose-ranging effect was shown with 100 billion c.f.u. yielding superior outcomes and fewer gastrointestinal conditions compared to 50 billion c.f.u.

#### ACKNOWLEDGMENTS

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#### CONFLICT OF INTEREST

**Guarantor of the article:** Xing Wang Gao, MD.

**Specific author contributions:** Planning study: Xing Wang Gao, Mohamed Mubasher, Chong Yu Fang, and Cheryl Reifer; conducting study: Xing Wang Gao, Chong Yu Fang, and Cheryl Reifer; collecting data: Xing Wang Gao and Chong Yu Fang; interpreting data: Xing Wang Gao, Mohamed Mubasher, and Larry E. Miller; drafting paper: Xing Wang Gao, Mohamed Mubasher, Cheryl Reifer, and Larry E. Miller; approval of submitted final draft: Xing Wang Gao, Mohamed Mubasher, Chong Yu Fang, Cheryl Reifer, and Larry E. Miller.

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**Potential competing interests:** None.

#### Study Highlights

##### WHAT IS CURRENT KNOWLEDGE

- ✓ Antibiotic-associated diarrhea and *Clostridium difficile*-associated diarrhea are common in hospitalized adults.
- ✓ Current treatments have limited effectiveness.

##### WHAT IS NEW HERE

- ✓ Probiotic prophylaxis lowers risk for antibiotic-associated diarrhea and, in particular, *Clostridium difficile*-associated diarrhea.
- ✓ Probiotic efficacy improves in a dose-dependent manner.

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