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Probiotics for *Clostridium difficile* infection in adults (PICO): Study protocol for a double-blind, randomized controlled trial

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Abstract

Background—*Clostridium difficile* is a pathogen of rapidly increasing public health importance.

An estimated quarter of a million *Clostridium difficile* infections (CDI) occur in the United States annually, at a resultant cost of 14,000 deaths and 1 billion dollars. *Clostridium difficile* related

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Author's Contributions

AB Drafted and edited manuscript.

MD Drafted and edited microbiology methods.

SV Developed inclusion and exclusion criteria, edited protocol. LAP critically edited the manuscript and participated in study design
RG critically edited the manuscript and provided guidance regarding study design NS Drafted and edited protocol, edited manuscript.

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Status of trial

The PICO trial is currently ongoing and is in the close-out phase.

Competing Interests

The authors have no conflict of interest to disclose.

deaths have risen 400% over the last decade, and current standard antibiotic treatments are only 75 to 85% successful. Besides increasing the risk of antibiotic resistance and side effects, these treatments are very expensive. The most vulnerable population for *Clostridium difficile* is older adults, who make up approximately half of the cases, but account for 90% of the related deaths. Probiotics may have potential as adjunctive therapeutic agents for CDIs, however, current data is limited.

Methods—This pilot study is a single-site, randomized, placebo-controlled, double-blind, phase two clinical trial. The trial primarily evaluates the effect of four weeks of probiotic therapy in addition to standard of care on *Clostridium difficile* diarrhea duration and recurrence. Secondary outcomes include effect on fecal cytokines, fecal lactoferrin, and *Clostridium difficile* toxin density in stool, as well as patient functional status.

Discussion—This pilot study will determine the feasibility and effect size to conduct larger randomized controlled trials of probiotic interventions in patients with CDI, to determine the impact of probiotics on the symptoms of CDI.

Keywords

Clinical trial; *Clostridium difficile*; diarrhea; Lactobacilli; Bifidobacteria; probiotics

Introduction

Clostridium difficile (*C.difficile*) is a pathogen of rapidly increasing public health importance. In 2013, the Centers for Disease Control and Prevention (CDC) labeled it as one of three “urgent threat” pathogens. This is the highest level possible, given to species that are “an immediate public health threat that requires urgent and aggressive action [1].” The CDC estimates that 250,000 *C. difficile* infections (CDI)¹ occur in the US annually, at a resultant cost of 14,000 deaths and 1 billion dollars [1]. This reflects a 400% increase in *C. difficile* related deaths between 2000 and 2007 [2], and a 25% higher incidence rate than methicillin resistant *Staphylococcus aureus* [3]. The most vulnerable population for *C. difficile* is older adults, who make up approximately half of CDI cases, but account for 90% of related deaths [2].

Subsequent recurrence of infection is dangerous and common for patients with *C. difficile*. Recent studies show that 25-30% of patients with an episode of CDI will experience recurrence [4-6]. The rates are even higher in adults over 65 years, who remain symptomatic with diarrhea for longer than younger patients, and suffer greater debility following a CDI episode [7,8]. Preventing CDI recurrence is essential, however, current strategies to hasten recovery and reduce recurrence are limited.

The most commonly prescribed method to treat CDI involves systemic antibiotic therapy with oral vancomycin or metronidazole [9]. During the past decade, success with these treatments has ranged from 64%-82% [10]. However, oral antibiotic therapy carries the risk of promoting antibiotic-resistant organisms or increasing relapse rates by further disrupting

¹Abbreviations: CDI: *Clostridium difficile* infection; DMC: Data Monitoring Committee; PICO: Probiotics for *Clostridium difficile* infection in adults.

the microbiome. Older adults are at elevated risk for medication intolerance or side effects [11]. Furthermore, oral vancomycin, increasingly the medication of choice for moderately severe CDI, is extremely expensive [12]. A newer antibiotic, fidaxomicin has a success rate similar to vancomycin, but is even more costly [13]. For alternative treatments, a single study of monoclonal antibodies has showed promising results [14]. However, their use in clinical care is limited based on their expense, their injectable form, and the fact that they are not commercially available. Probiotics represent a potentially promising approach to preventing recurrent CDI, by enhancing gut-associated immunity and ameliorating the gut microbiota imbalance that is a hallmark of CDI [15]. We hypothesize that probiotics, in conjunction with standard antibiotic therapy, will reduce the duration of diarrhea, reduce recurrence, and improve functional status in adults with initial episode of CDI. If probiotics are found to reduce recurrence, patients will experience fewer subsequent CDIs. Since antibiotics are standard therapy for CDI treatment, reducing the rate of future CDIs could greatly curtail the use of conventional antibiotics among these patients, thus providing substantial individual and societal benefit.

We will conduct a pilot randomized, double-blinded, placebo-controlled, phase two clinical trial in patients with a first episode of CDI, primarily to evaluate the efficacy of probiotic treatment for reducing the duration of diarrhea and CDI recurrence. We evaluate the effect that the probiotic use has in reducing the density of *C. difficile* toxin in stool, stool cytokines, and fecal lactoferrin, as well as its role in improving the functional ability of recovering patients. Given its rapidly increasing prevalence, addressing CDI could have profound physical and functional benefits.

Materials and Methods

Study design

This is a pilot randomized, double blinded, placebo controlled, phase two clinical trial in adult patients with a first episode of CDI. It is designed to test the hypothesis that compared to standard care and placebo, daily oral probiotic as adjunctive treatment for CDI will reduce the duration of diarrhea and the incidence of CDI recurrence. Both the experimental and control groups receive antibiotics for treatment of CDI as outlined in recent multinational guidelines for management of CDI [16,17]. Enrollment in the trial is from February 2013 to February 2015.

Rationale

Probiotics have several effects which may promote improved immune system functioning. These include modulating the gut microbiota and interacting with the innate and adaptive immune systems of the host [18-21]. At the level of the intestinal epithelium, probiotic strains also preserve or strengthen the mucosal gastrointestinal barrier by prevention of bacterial adherence to the epithelial lining. This occurs through competitive exclusion, inhibition of pathogenic induced alterations of epithelial permeability, and regulation of enterocyte gene expression involved in maintenance of the mucosal barrier.

We are using a combination probiotic of 4 bacterial strains as the intervention in our study. Human studies confirm the immunostimulatory effects of the probiotics to be used in this study. Three of the four strains in the probiotic combination—*Lactobacillus acidophilus* (*L. acidophilus*) NCFM®, *Bifidobacterium lactis* (*B. lactis*) BI-04, and *B. lactis* BI-07—have been shown to stimulate human response to vaccination, and supplementation with BI-07 was shown to significantly increase serum immunoglobulin G levels [22]. A probiotic combination in which *Lactobacillus paracasei* (*L. paracasei*) Lpc-37 was the main component has been shown to significantly increase phagocytic activity of monocytes and granulocytes in healthy subjects [23]. Finally, *L. acidophilus* NCFM® has been shown to reduce cold and flu duration in children [24], alter allergic rhinitis-associated biomarkers [24], and enhance cellular immunity in older adults [25].

Gastrointestinal tract survival and intestinal adhesion of probiotics

Survival during passage through the host gastrointestinal tract, including acidic and alkaline environments, is essential for the successful activity of probiotics. All four probiotic strains used in this study are able to survive acidic concentrations equivalent to those of the stomach, and concentrations of bile salts equivalent to those of the duodenum. Additionally, *L. acidophilus* NCFM, *B. lactis* BI-04, *B. lactis* BI-07, and *L. paracasei* Lpc-37 have been detected in host feces in human trials, providing further evidence that the probiotic strains comprising the probiotic combination can survive passage through the host gastrointestinal tract [23,26,27].

Adhesion to the intestinal mucosa is also a prerequisite for successful probiotic activity. The benefits of *L. acidophilus* NCFM®, including intestinal and oral adhesion, have been demonstrated in animal and human studies. *In vitro* studies have demonstrated good adhesion of *B. lactis* BI-04, *B. lactis* BI-07, and *L. paracasei* Lpc-37 to human epithelial cell lines (Internal data, Danisco).

Participant selection

The inclusion and exclusion criteria for subject enrollment are summarized in Figure 1.

Inclusion criteria—We screen adult patients diagnosed with a first episode of CDI at the University of Wisconsin Hospital and Clinics. Adults include all patients 18 years or older, of either gender. An episode of CDI is defined by diarrhea associated with a positive stool test for *C. difficile* toxin(s) in the 2 days prior to enrollment, as confirmed by reviewing electronic medical record data.

Exclusion criteria—Patients are excluded from study if they have severe CDI, as defined by intensive care unit placement at the time of CDI diagnosis, white blood cells > 30,000 or < 1000 cells/mm³, elevated creatinine > 1.5 times the premorbid level, or toxic megacolon [28, 29]. Other exclusion criteria include prior history of CDI, another known etiology of diarrhea (e.g., other enteric pathogen, other intestinal disease), a history of chronic intestinal disease (e.g., Crohn's disease, ulcerative colitis), the presence of ileus, colostomy, gastric tube, or naso-gastric tube, and a history of abdominal surgery within the 3 months prior to study enrollment. Patients are also excluded if they are currently enrolled in another

investigational drug trial, unavailable for follow-up visits, or not willing to stop using other probiotics for the duration of the study. In addition, patients are excluded if they are severely immunocompromised, as defined by either having the diagnosis of HIV, AIDS, or a primary immunodeficiency, or having a very recent solid organ or bone marrow transplant.

Although probiotics have been used safely in studies with pregnant women, known pregnancy is another exclusionary criterion for enrollment in our study. We do not require a pregnancy test before study enrollment, however if a woman determines that she is pregnant during the study period, she must stop the study medication. She will continue other procedures as listed in the protocol.

Recruitment

Patients with CDIs at the University of Wisconsin Hospital and Clinics are identified through daily review of microbiology reports. The University of Wisconsin Hospital is a 536 bed tertiary care academic medical center. From these participants, patients with a first episode of CDI are identified by a review of their electronic medical records. Patients with a mild to moderate first episode of CDI who meet the inclusion and exclusion criteria are recruited in person in an inpatient setting. To increase recruitment opportunities and advertise the study, and email was sent out to all healthcare providers that are involved in the collaborating clinics. Informational flyers were also placed in each collaborating clinic. In both ward and clinic settings, a member of the research team provides patients with details about the study and obtains the subject's informed consent prior to enrollment. All study procedures and informed consent documents have been approved by the University of Wisconsin-Madison institutional review board (IRB). Consent documents are available on request from the communicating author. All protocol modifications are communicated to the institutional review board by the Principal Investigator.

Compensation

Two \$50 checks are provided as study compensation, for a total compensation of \$100. One check is given for the completion of Study Visit 1 (Week 0) and the second check for completion of Study Visit 3 (Week 8) at the end of study. A subject has to complete all three study visits to receive the entire amount.

Randomization

Our trial is double blinded, using an identical matching placebo. Both the subjects and research team are blinded. For the study investigators to remain blinded, assignment to study intervention or placebo takes place centrally at the University of Wisconsin research pharmacy using a random-number generator. The research pharmacy has no stake in studies, and specializes in systems to promote allocation concealment. Treatment allocation is by random assignment in a 1:1 ratio in permuted blocks of 4. Since other studies have found that the recurrence rate is similar with vancomycin and metronidazole and the severity of illness is not closely related to recurrence, we have not stratified by these additional factors [30]. The unit of randomization is the individual.

Intervention

The intervention is the administration of a probiotic combination. The subject takes the oral probiotic or placebo every day for 28 days. After week four, the subjects ceases taking the probiotic or placebo. All subjects are followed for CDI recurrence until week eight. All also receive the standard antibiotic treatment for CDI, with dosing as directed by their treating physician. The use of other probiotics is prohibited during the trial.

Probiotic—The oral probiotic, consisting of *L. acidophilus* NCFM® (ATCC 700396), *L. paracasei* Lpc-37 (ATCC SD5275), *B. lactis* Bi-07 (ATCC SC5220), and *B. lactis* B1-04 (ATCC SD5219), is administered once daily in capsule form at a dose of 1.70×10^{10} CFU. The probiotic is provided by the manufacturer, Danisco (Madison, WI). Random samples of the study drug are cultured once monthly to ensure that colony counts remain stable. The probiotic is administered in addition to a standard duration (10-14 day) course of the standard antimicrobial therapy for a first episode of CDI, vancomycin or metronidazole.

Placebo—The placebo is identical to the study treatment in appearance and taste, and contains the inert filler that the active product also contains. The manufacturer, Danisco, supplies the placebo. The placebo is also administered in addition to a standard duration course of standard antimicrobial therapy for a first episode of CDI.

Intervention timeline

A study diagram is shown in Figure 2, and intervention details are outlined below.

Study Visit 1—At the first visit, week zero, the subject is enrolled following informed consent, and randomized to probiotic or placebo. An initial stool sample is collected. During hospitalization, subjects are followed daily for diarrhea history, record of concomitant medications, adverse effects, and their response to treatment using a stool diary.

Upon discharge, subjects are followed through weekly clinic visits or telephone calls to identify continuing symptoms of CDI, side effects from the medication, and any problems taking the medication. Functional status is assessed by the Barthel Index [31]. All subjects are given a stool diary card to report diarrhea history. A modified version of the Bristol stool consistency scale is used for the assessment of diarrhea [32]. The subjects fill out the stool diary daily for 8 weeks, beginning at enrollment.

Home collection kits are also given at visit 1, and subjects are trained in stool collection. Subjects are asked to bring their stool samples to visits 2 and 3. Sample collection is allowed within 4 days of the expected date, to coincide with clinic visits. Stool samples are collected in a sterile container, and brought or mailed to the clinic.

Study Visit 2—After 28 days, the subject stops taking the probiotic or placebo. A stool sample is collected, and the stool diary is reviewed for diarrhea duration and CDI recurrence.

Study Visit 3—At week 8, a final stool sample is collected, and the stool diary is reviewed for CDI recurrence.

If subjects have signs or symptoms of recurrent CDI at any point during the study after resolution of the initial episode, an additional stool sample is collected. If a subject is diagnosed with recurrent CDI, they will be discontinued from study treatment and treated according to standard of care for recurrent CDI.

Adherence

We measure medication adherence using the Medication Event Monitoring Systems (AARDEX Ltd, Switzerland) cap data, pill counts at the week 4 visit, and self-reporting at weekly phone calls. Tolerability is an important factor in subject adherence to a potentially beneficial intervention. The four strains proposed for this study have all demonstrated good tolerability in human subjects.

Subject withdrawal

All subjects are informed during enrollment that they may discontinue participation at any time. We ask that they contact the study coordinator if they decide to drop-out of the study. If the subject is willing, the research coordinator arranges a research clinic visit to collect a final stool sample and stool diary charts. Intention to treat analysis will be performed on all patients lost to follow up.

Outcomes

We hypothesize that modulation of the gastrointestinal flora and enhancement of systemic immune functioning by a probiotic combination consisting of equal amounts of *L. acidophilus* NCFM®, *B. lactis* BI-04, *B. lactis* Bi-07, and *L. paracasei* Lpc-37 will lead to a two-day reduction in the duration of diarrhea, and a reduction in CDI recurrence, as compared to placebo and standard care. The beneficial effects may be mediated by competitive exclusion (gastrointestinal colonization) and immunostimulation (nasal colonization). While we are not studying all the potential mechanisms of probiotics, the two we have chosen to include are the most biologically plausible and the most relevant to *C. difficile* infection.

We define diarrhea as the presence of three or more loose stools in a 24 hour period [33]. Stool diaries are collected daily from baseline for 8 weeks. We define “loose stools” as levels 1-4 on our in-house stool scale, as self-recorded in subject's diary. This visual stool scale is provided to all patients at enrollment, and available from the corresponding author upon request. Levels 1-4 correspond to Bristol stool levels 5-7 [32]. All subjects will be evaluated for duration of diarrhea: the time period between when diarrhea started, and when less than 3 loose stools are reported in the stool diary. We hypothesize that the group treated with probiotics will have a two-day reduction in the duration of diarrhea, compared to the placebo group.

CDI recurrence is evaluated for all patients who re-experience symptoms after successful treatment of their initial CDI. Recurrence is defined as the presence of *C. difficile* toxin in the stool, after successful treatment of an initial CDI. The presence of *C. difficile* toxin is assessed by an enzyme immunoassay, as discussed in microbiologic analysis section below. Recurrence can take two forms, relapse of the initial infection and reinfection by a new

strain. Relapse and reinfection is distinguished by analyzing *C. difficile* toxin in a stool sample submitted during the second symptomatic period. For relapse, subjects having a stool isolate identical to stool isolate(s) from the original infection is assessed by genotyping via Pulse Field Gel Electrophoresis. The episode is considered reinfection if the strains of CDI are found to not be related. Given previous studies in the literature, we anticipate that relapse will be more common than reinfection [34].

As secondary outcomes, we hypothesize that compared to placebo, probiotic treatment will significantly decrease the density of *C. difficile* toxin in the stool, fecal cytokines, and fecal lactoferrin, and improve the functional ability of recovering patients. Measurement of these outcomes are described below.

Microbiologic analysis

Isolation of fecal anaerobic bacteria—We hypothesize that the competitive exclusion of pathogens in the gastrointestinal tract occurs by an increase in normal fecal microbiota. With the probiotic, we expect to see increases in fecal lactic acid bacteria compared with subjects receiving placebo. Probiotics may also have effects on the non-lactic acid bacteria of the gut, and thus stool processing for major categories of anaerobes is being undertaken using microbiome analysis. We expect that in the group receiving probiotics, there will be a greater preponderance of the lactobacilli compared with placebo.

Assessment of probiotic presence in fecal samples—0.1 grams of fresh stool is added to 0.9 mL of sterile phosphate buffered saline (PBS) and diluted to 10^{-6} . 100 μ L dilutions aliquots of 10^{-3} , 10^{-5} , and 10^{-6} dilutions are plated onto MRS Lactobacillus Agar. Plates are incubated at 36°C for 48 hours anaerobically (10% CO₂, 10% H₂, 80% N). Colonies representative of *Lactobacillus* species are isolated. The isolates are gram stained, and a catalase test is performed. Gram positive rods that are catalase negative are stocked for further workup: species and strain specific PCR are run on all Lactobacillus isolates.

***C. difficile* toxin, lactoferrin and cytokines**—A stool sample is collected on all subjects at baseline, 4 weeks, and 8 weeks, to determine the presence of *C. difficile* in qualitative stool cultures, using broth enrichment followed by culture on *Clostridium difficile* Brucella Agar. Toxin quantitation is done using an ELISA methodology (tgcBIOMICS). Fecal cytokines are assayed using an ELISA. The following cytokines are examined: CXCL-5/ENA-78, and CXCL-8/IL-8 (Quantikine R&D Systems, Minneapolis, MN). This kit is validated for feces using a protocol obtained from R&D Systems. Fecal lactoferrin are measured by the commercial IBD-Scan test (TechLab, Blacksburg, VA) in a quantitative manner.

Adverse effects

Safety of probiotics—*L. acidophilus* NCFM® has performed as a safe probiotic agent in animal and human studies. It was made commercially available 30 years ago, has been widely available in the North American market for over 15 years, has a long history of safe performance in humans. Safety concerns related to probiotics include their potential for causing bacteremia and transferring antibiotic-resistant genes. In a 10-year survey conducted

in Finland, no incidents of bacteremia due to *L. acidophilus* NCFM[®] were found [35]. Clinical infections, including bacteremias, due to *Lactobacillus* species most often arise from the patient's endogenous microbiota. The majority of clinical infections due to lactobacilli species from probiotics occur in immunocompromised or severely ill patients, which we have excluded in our study. Finally, genomic analysis has found none of the genetic elements known to be associated with transfer of antibiotic resistance to be present in *L. acidophilus* NCFM[®] [36].

Bifidobacterium species have long been considered safe for human consumption, and their safety has been studied in many publications [37, 38]. Clinical trials have demonstrated the safety of *B. lactis* BI-04 and *B. lactis* BI-07 for consumption by children, adults, and elderly subjects. No acquisition of antibiotic resistance was detected in *B. lactis* BI-04 or *B. lactis* BI-07 by the European Union funded PROSAFE screening project.

Safety of placebo—The placebo agent will be composed of microcrystalline cellulose, an inert, inactive substance. Being an inert substance, the likelihood for adverse reaction is virtually none, and there are no obvious foreseeable risks associated with the ingestion of the placebo capsules.

Safety Monitoring—During hospitalization, daily assessment of fever and other signs of infections are made in person by a member of the research team. Following discharge, subjects are advised to self-monitor for fever, any other signs of infections, and any treatment side effects. They are subsequently followed on a weekly basis by the research team through telephone calls. If evidence of clinical infection is apparent, the subject seeks care with their physician provider and blood cultures are collected. The incident is reported to the IRB and Data Safety Monitoring Committee. If the blood cultures are positive for one or more of the probiotic stains, pulsed-field gel electrophoresis is conducted to examine relatedness. If the subject is found to be in the treatment group, the probiotic therapy will be immediately stopped. Clinical infections will be promptly treated with appropriate antibiotics and managed based on the severity of illness.

If an adverse or severely adverse event occurs, timely, accurate and complete reporting and analysis of safety information will be undertaken. The following events will be reported as serious adverse events: An increase from normal laboratory values at baseline to the modified National Cancer Institutes criteria of Grade 3 or more, an increase of laboratory values from modified National Cancer Institutes criteria grade 1 or 2 at baseline to grade 4, a new cancer diagnosis, and medical events of interest [39].

Data Management

Research data is handled with utmost confidentiality and discretion. Subjects are assigned a unique identification number that can be traced only by the research specialist and PI. The file linking study identification numbers to identifiable information is stored and secured separately from the coded data. All subject information is kept in locked drawers, file cabinets or secure computer files, with access only allowed to research personnel. The final trial dataset will be available to all research personnel.

Data safety monitoring is performed by an independent Data Monitoring Committee (DMC) of the University of Wisconsin at Madison Institute for Clinical and Translational Research. The DMC ensures subject safety, research data integrity, and compliance with federal regulations in the proposed research. The DMC also preforms periodic auditing of trial conduct, and makes recommendations that could include actions of continuation, modification, or termination.

Statistical analysis

Sample size justification—We are conducting a randomized controlled trial with probiotic and placebo randomized in a 1:1 manner. This is a pilot feasibility study. We hypothesize that probiotic therapy will reduce the mean duration of diarrhea by two days. Estimates from the literature would suggest that in our elderly population the average duration of diarrhea is 10.2 days with a standard deviation of 2.32 days when treated with the standard antibiotic regimen [40, 41]. Using a two-sided nonparametric Wilcoxon rank-sum test with a 0.05 significance level, an N of 23 subjects per group is needed to achieve 80% power to be able to detect a reduction of 2 days. Adjusting for a 20% drop out rate increases the final sample size to a total of 58 subjects, 29 in each arm. Therefore, the target population will be 58 adults aged 18 years or older, over a period of two years. Because this is a pilot feasibility study, a sample size calculation for the second primary end point recurrence was not conducted.

Data analysis—Following descriptive statistical analyses, and assessment of missing data, intention to treat analysis will be used. Duration of diarrhea will be compared between treatment and control groups using a Wilcoxon rank-sum test, and analysis of covariance will be used to adjust for gender and other relevant covariates. Chi-square test will be used to compare the recurrent CDI rate between treatment and control groups, and logistic regression will be used to adjust for gender and other relevant covariates. Density of *C. difficile* toxin, fecal cytokines and lactoferrin will be compared using Wilcoxon rank-sum test, using log transformation if necessary, and analysis of covariance will be used to adjust for covariates as necessary. Analysis of fecal cytokines is exploratory and levels will be compared among treatment groups using Wilcoxon rank-sum test. The Barthel index is exploratory and scores will be compared within and between subjects.

Discussion

This study will identify the major mechanisms by which probiotics are expected to mediate their effect on CDI symptoms and recurrence. In turn, shortened duration of CDI and decreased recurrence will help to reduce the disease burden of *C. difficile*. Probiotics offer the potential for safe, low cost, non-antibiotic adjunct therapy to standard treatment regimens. By studying probiotics in a population that includes older adults, we hope to shed new light on the treatment of *C. difficile* in a high-risk population, in which CDIs are disproportionately fatal.

The primary limitation of this study is the sample size. This was designed as a pilot study, and the recruitment goal over a two year period is 58 patients with mild to moderate *C.*

difficile. This trial is planned to assess feasibility, attrition, completion of study procedures, and recruitment challenges, prior to undertaking a larger study.

Because this study is being conducted at a single site, recruitment is particularly vulnerable to changes in local CDI patterns. The study site is working diligently to decrease rates of hospital acquired CDI. If these infection prevention efforts are successful, or the enrollment rate for eligible subjects is less than anticipated, it may be difficult to meet our recruitment goal during the two year period. Furthermore, limiting the study to non-severely immunocompromised, non-ICU patients without a prior history of CDI, excludes high CDI risk populations, and is another potential recruitment challenge.

Finally, by excluding subjects with severe CDIs, we are unable to investigate the role of probiotics for preventing *C. difficile* recurrence in these patients. Patients affected by severe CDIs are at a higher risk for clinical complications, often have longer recovery times, and may require more aggressive therapy. People with severe CDI would benefit greatly from a reduction in the rates of CDI recurrence, and it is important to include these patients in future studies.

If, as expected, CDI symptoms, biomarkers of inflammation, or recurrence is reduced, the next step is to undertake an adequately powered, multisite study to examine the efficacy of probiotics for reducing CDI symptoms and recurrence in the larger population. Comparative effectiveness research, comparing the impact of probiotics to currently available adjunctive treatments for CDI is the logical extension of our work. Future studies may also examine the impact of probiotics on colonization and infection by other multidrug-resistant bacteria, such as vancomycin-resistant enterococcus.

Ultimately, this study will produce data, methods, and tools that have widespread relevance and portability, and have the potential to reduce healthcare associated infections.

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Inclusion Criteria
Adults of either gender, > 18 years old with a first episode of CDI.
Meets the case definition of CDI: Diarrhea associated with a positive stool test for <i>C. difficile</i> toxin(s) in the 2 days prior to enrollment.
Exclusion Criteria:
Severe disease defined as any of the following: White blood cells > 30,000 or < 1000 cells/mm ³ , elevated creatinine > 1.5 times the premorbid level, or toxic megacolon.
Intensive care unit placement at time of CDI diagnosis.
Prior history of CDI.
Other known etiology of diarrhea (e.g., other enteric pathogen, other intestinal disease).
History of chronic intestinal disease (e.g., Crohn's disease, ulcerative colitis).
Presence of ileus, colostomy, gastric-tube, or naso-gastric tube.
History of abdominal surgery within the previous 3 months.
Enrolled in another investigational drug trial.
Unavailable for follow-up visits.
Not willing to stop using other probiotics.
Severely immunocompromised (HIV, AIDS, primary immunodeficiency, very recent solid organ or bone marrow transplant).
Known pregnancy.

Figure 1.
Inclusion and exclusion criteria for subject enrollment.

Figure 2.

Schematic representation of PICO study. Week 0: Recruit, consent, enroll, randomize. Week 0-4: Intervention. Week 4-8: Washout, monitor recurrence.