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A randomized controlled trial of probiotics for *Clostridium difficile* infection in adults (PICO)

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Background: *Clostridium difficile* is the most common cause of hospital-acquired infections, responsible for >450000 infections annually in the USA. Probiotics provide a promising, well-tolerated adjunct therapy to standard *C. difficile* infection (CDI) treatment regimens, but there is a paucity of data regarding their effectiveness for the treatment of an initial CDI.

Objectives: We conducted a pilot randomized controlled trial of 33 participants from February 2013 to February 2015 to determine the feasibility and health outcomes of adjunct probiotic use in patients with an initial mild to moderate CDI.

Methods: The intervention was a 28 day, once-daily course of a four-strain oral probiotic capsule containing *Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium lactis* Bi-07 and *B. lactis* Bl-04. The control placebo was identical in taste and appearance. Registered at clinicaltrials.gov: trial registration number = NCT01680874.

Results: Probiotic adjunct therapy was associated with a significant improvement in diarrhoea outcomes. The primary duration of diarrhoea outcome (0.0 versus 1.0 days; $P = 0.039$) and two exploratory outcomes, total diarrhoea days (3.5 versus 12.0 days; $P = 0.005$) and rate of diarrhoea (0.1 versus 0.3 days of diarrhoea/stool diary days submitted; $P = 0.009$), all decreased in participants with probiotic use compared with placebo. There was no significant difference in the rate of CDI recurrence or functional improvement over time between treatment groups.

Conclusions: Probiotics are a promising adjunct therapy for treatment of an initial CDI and should be further explored in a larger randomized controlled trial.

Introduction

Clostridium difficile is implicated in over 450000 infections and 15000 deaths annually in the USA.[1] Treatment of *C. difficile* infection (CDI) is a major challenge. Approximately 25%–30% of patients treated for a primary CDI experience recurrence.[2–4]

The opportunistic nature of *C. difficile* allows it to proliferate and cause infection in the gastrointestinal (GI) tract during microbial disruption. Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts.[5,6] Probiotic therapies pass through the host GI tract while harbouring a spectrum of potentially protective benefits.[7–10]

Meta-analyses of randomized controlled trials (RCTs) have demonstrated that ingestion of specific probiotic strains during antibiotic therapy significantly reduces the risk of developing a CDI.[11–14] However, studies evaluating the effectiveness of probiotics as treatment during an initial CDI episode are lacking. Thus, we undertook a pilot RCT to determine the feasibility and health outcomes of adding daily probiotic as an adjunct therapy to standard antibiotic treatment for participants with an initial CDI. We hypothesized that, compared with placebo, adjunct probiotics would lead to a reduction in the duration of *C. difficile* diarrhoea and reduce the likelihood of CDI recurrence.

Methods

This Phase 2, pilot, RCT is registered with clinicaltrials.gov (NCT01680874) and approved by the Health Sciences Institutional Review Board at the University of Wisconsin (UW) Madison. Study enrolment took place at UW Hospital, a 536 bed tertiary care academic medical centre, from February 2013 to February 2015. The study protocol for this trial was published previously.[15]

Study participants

In accordance with inclusion criteria, all study participants were adults experiencing an initial episode of mild to moderate CDI. Exclusion criteria included severe CDI, prior history of CDI, other known aetiology of diarrhoea, history of chronic intestinal disease, abdominal surgery in the past 3 months, presence of ileus, colostomy, gastric or nasogastric tube, a severely immunocompromised state, pregnancy, unavailability for follow-up, unwillingness to terminate other probiotic use, or enrolment in another investigational drug trial.[15] Patients were randomized using a random-number generator in a 1:1 ratio in permuted blocks of four. All patients provided informed consent prior to enrolment.

Intervention

All participants received a placebo or single multi-strain oral probiotic capsule (*Lactobacillus acidophilus* NCFM, ATCC 700396; *Lactobacillus paracasei* Lpc-37, ATCC SD5275; *Bifidobacterium lactis* Bi-07, ATCC SC5220; *B. lactis* Bl-04, ATCC SD5219; 1.70×10^{10} cfu per capsule) daily for 4 weeks. The pills were identical in appearance and taste.

Timeline

During the trial, all participants continued standard CDI antibiotic treatment. Clinic visits occurred at weeks 0, 4 and 8. Participants submitted a fresh stool sample and daily stool diary, modified from the Bristol Stool Consistency Scale,[16] at those timepoints. Weekly telephone calls were conducted to evaluate treatment adherence, adverse effects, ongoing symptoms and functional status, assessed by the Barthel Index of Activities of Daily Living.[17]

Outcomes

Two primary outcomes were duration of diarrhoea and CDI recurrence. Diarrhoea was defined as three or more loose stools in 24 h. Duration of diarrhoea was the number of consecutive 24 h periods with diarrhoea that occurred after study enrolment, beginning on the first stool diary day. Recurrence was defined as the presence of toxigenic *C. difficile* in the stool of a symptomatic participant, who had previously cleared their CDI. There were two secondary outcomes: functional status (weeks 4 and 8) and total adverse events (week 8).

Two exploratory diarrhoea outcomes were defined after completion of data collection, but prior to unblinding the treatment allocation scheme. These include the total number of days that participants experienced diarrhoea and the rate of diarrhoea. These measurements were introduced due to high rates of incomplete stool diary data, which made it desirable to conduct an analysis of diarrhoea events using the denominator of person-time. Missing data was defined as three of more consecutive days without a recorded stool diary entry. If 1 or 2 days passed without a stool diary entry, we assumed that the participant did not have a bowel movement during this time and it was not considered missing. The rate of diarrhoea was calculated as the total number of diarrhoea days divided by the total number of non-missing stool diary days.

Statistical analysis

All statistical analyses of main outcomes were performed with the SAS software (version 9.4; Cary, NC, USA) using ITT methodology. Comparisons between the treatment arms were conducted using the Wilcoxon rank-sum test, the χ^2 test or Fisher's exact test with a significance level of 0.05.

Results

Of the 590 potential participants assessed for eligibility, 43 met inclusion and exclusion criteria and 33 enrolled (76.7% response rate). Two participants, one from each treatment group, were lost to follow-up before they submitted any stool diary data. Thus, the sample size was 31 for all primary and exploratory diarrhoea outcomes except for CDI recurrence. Among the 31 participants who submitted stool diary data, 10 reported <7 weeks of data, with an average among these participants of 31.5 non-missing days. Recurrence was evaluated in 28 participants who recovered from their CDI during the study and submitted follow-up stool samples. Baseline characteristics of probiotic and placebo groups were similar (Table 1).

The median duration of diarrhoea was 1.0 day longer in the placebo group than the probiotic group ($P = 0.039$; Table 2). The difference in recurrence was not significant (Table 2). Both exploratory diarrhoeal outcomes, total diarrhoea days and rate of diarrhoea, were significantly worse for participants treated with placebo compared with participants treated with probiotics (Table 2).

There was no significant difference between the groups concerning participants' functional ability at week 4 or 8 (Table 2) or total number of adverse events. Almost all (96.8%) participants experienced at least one adverse event. GI disorders were the most common (75% probiotic, 80% placebo).

Discussion

Combination probiotic treatment was associated with significant improvement in diarrhoea outcomes for participants, compared with placebo. Shortening the duration of an initial CDI could allow patients to stop antibiotic therapy sooner, having considerable downstream implications for reducing antibiotic resistance.

To our knowledge, no other studies have investigated probiotics as adjunct therapy for initial CDIs. Existing studies focused on probiotics for the prevention of initial and recurrent CDIs and recurrent CDI treatment. Transient probiotic GI colonization is a key component of the biological mechanism reducing CDI in all these clinical situations. Our findings trend in the same direction as recent meta-analyses evaluating probiotic RCTs in the other *C. difficile* contexts.[11,13,14] However, the effects of probiotics are known to be strain-specific and current guidelines from the IDSA classify probiotic therapy as an unresolved issue requiring further study.[18]

Future evaluations of the use of this combination probiotic are warranted. Recruitment proved difficult at our single study site. The primary barrier was stringent inclusion and exclusion criteria. Small sample size is a limitation of this study. Type I error is particularly concerning for low powered studies and it is possible that our findings do not reflect a true difference between treatment groups. However, it is reassuring that the results are consistent across outcome measures. In future studies, utilization of multiple study sites or a longer study duration would allow for the recruitment of a larger sample size.

This pilot study revealed feasibility concerns regarding stool diary recording. We added a diarrhoea rate outcome to

Table 1. Patient demographics and health status at enrolment

Variable	Placebo, $N = 15$	Probiotic, $N = 16$	P
Demographics			
age (years), median (IQR)	57.0 (42.0–73.0)	65.0 (42.5–70.5)	0.51
race, *n* (%)			0.37
white	13 (87)	15 (94)	
other	2 (13)	1 (6)	
female, *n* (%)	11 (73)	11 (69)	0.78
C. difficile treatment			
primary antibiotic treatment, *n* (%)			0.72
vancomycin	9 (60)	8 (50)	
metronidazole	6 (40)	8 (50)	
days of antibiotic treatment prior to enrolment, median (IQR)	3.0 (1.0–5.5)	2.0 (1.0–3.3)	0.16
Baseline health status, *n* (%)			
shortness of breath	3 (20)	2 (13)	0.57
angina	1 (7)	1 (6)	0.96
muscle aches	3 (20)	4 (25)	0.67
prior GI surgery	9 (60)	7 (44)	0.37
cough	2 (13)	7 (44)	0.06
diarrhoea	15 (100)	15 (94)	
nausea	5 (33)	4 (25)	0.60
frequent urination	0 (0)	0 (0)	
painful joints	5 (33)	4 (25)	0.69

Table 2. Study outcomes regarding diarrhoeal symptoms, CDI recurrence and Barthel Index functional score

Variable	Placebo, $N = 15$	Probiotic, $N = 16$	P
Duration of diarrhoea (cumulative days), median (IQR)	1.0 (0.0–13.0)	0.0 (0.0–2.0)	0.039
Diarrhoea rate (days of diarrhoea/stool diary days submitted), median (IQR)	0.3 (0.1–0.5)	0.1 (0.0–0.2)	0.009
Total number of days with diarrhoea (days), median (IQR)	12.0 (6.0–25.0)	3.5 (1.0–8.0)	0.005
Stool diary number of days, median (IQR)	55.0 (38.0–56.0)	57.0 (37.0–59.0)	0.55
CDI recurrence[a], *n* (%)			0.96
0	12 (92)	14 (93)	
1	1 (8)	1 (7)	
Total Barthel Index score, mean (SD)			
week 4	18.3 (2.7)	18.2 (3.4)	0.86
week 8	19.1 (3.2)	18.8 (2.5)	0.23

[a]$N = 13$ for placebo and $N = 15$ for probiotic.

account for the differences in person-time reported between participants. To minimize reporting bias, we employed a 2 day cut-off to differentiate between gaps in stool diary entries due to a lack of bowel movements versus incomplete reporting. Future studies could better engage participants using an electronic stool diary tool or linking study compensation to diary completion. Both were utilized effectively in a recent study of patients with irritable bowel that tracked 90 day stool histories.[19]

The duration of diarrhoea measurement was another limitation, as it could not capture diarrhoea that occurred before the first stool diary was recorded. The stool diary start date varied, with some participants recording in the hospital and others beginning at discharge. There was no systematic difference between groups concerning when diary recording started. This measurement should be standardized in future studies and include an assessment of inpatient hospital records.

Finally, by definition, recurrence could only be assessed among the subset of participants who cleared their initial CDI during the study period and provided follow-up stool samples. Bias due to disruption in the randomization scheme is minimized in this study, as only five participants were excluded from the analysis of CDI recurrence (three placebo, two probiotic). Future studies focused on recurrence should evaluate the effect of probiotics for prevention in a randomized population of patients, all of whom have already recovered from a CDI.

In this pilot study, a combination probiotic was found to decrease significantly the duration of CDI diarrhoea compared with a placebo control. Additional studies are needed to investigate this finding in a larger patient population, but these results are promising. Given the current burden of CDIs in hospitals, even a small decrease in diarrhoea duration for patients is poised to have a considerable impact on *C. difficile* transmission and antibiotic usage and warrants rigorous assessment in efficacy trials.

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The study protocol was published previously in the journal *Contemporary Clinical Trials*.[15]

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Transparency declarations

None to declare.

Author contributions

A. K. B. drafted and edited the manuscript. M. D. performed non-microbiome laboratory analyses and drafted and edited the microbiology methods. S. V. developed study inclusion and exclusion criteria and edited the original study protocol. T. H. performed data analyses and drafted the statistical analysis methods. L. A.-P. and R. G. participated in study design and edited the manuscript. N. S. participated in study design, drafted and edited the original study protocol and edited the manuscript.

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