

## Probiotics for recurrent *Clostridium difficile* disease

In this journal's recent review of *Clostridium difficile*-associated disease (CDAD) management, McFarland (2005) emphasized the need for definitive efficacy trials to test recurrent CDAD (RCDAD) therapies. To address this recommendation, we report results and feasibility issues learned from a pilot trial of adjunctive *Lactobacillus rhamnosus* GG (LGG) for prevention of RCDAD.

Adults presenting with RCDAD (i.e. diarrhoea, enzyme immunoassay detection of stool *C. difficile* toxin A or B, and history of CDAD in the preceding year) were recruited from two hospitals and an extended care facility in St. Louis, Missouri, USA, over 9 months. Exclusion criteria included critical or terminal illness, compromised immunity, prosthetic heart valves, >5 days of anti-*C. difficile* antibiotic therapy, recent probiotic use and confounding diarrhoeal illness. Informed consent was obtained from all participants or their proxies, and the study was approved by the institutional review board of each institution.

Participants were randomized to receive one LGG capsule (40 mg lyophilized LGG and 320 mg inulin) or one placebo capsule (360 mg inulin) orally twice daily, adjunctively with anti-*C. difficile* antibiotics as chosen by the primary clinician, for the duration of antibiotic therapy and for an additional 21 days. The LGG and placebo capsules were donated by CAG Functional Foods, Omaha, Nebraska, USA. Investigators and participants were blinded to the treatment assignment. Randomly selected LGG capsules were analysed and contained  $2.8 \times 10^{11}$  c.f.u. per capsule of Gram-positive bacilli with uniform colony morphology at study initiation and  $4.0 \times 10^{10}$  c.f.u. after 8 months stored at 4 °C. The primary outcome was subsequent RCDAD within 60 days of completing anti-*C. difficile* antibiotic therapy. Participants were analysed within their assigned treatment arm in an intention-to-treat analysis, using *t*-tests and Mann-Whitney U tests for continuous variables and Fisher's

Exact tests for categorical variables in Statistical Package for the Social Sciences version 12.0.

Among the 15 enrolled participants, the mean age was 77.9 years, 13 (86.7 %) were women, five (33.3 %) demonstrated cognitive dysfunction and the median number of prior CDAD episodes was 1.0 (maximum of three) (see Table 1). Eight participants (53.3 %) received LGG and were similar to placebo recipients with respect to demographic and clinical features. Three (37.5 %) cases of RCDAD were observed in the LGG arm and one (14.3 %) in the placebo arm [risk ratio 2.6 (95 % confidence intervals 0.3–19.9)]. The median duration of the anti-*C. difficile* antibiotic regimens was 18.0 days and similar between arms; 12 (80 %) of the participants received metronidazole monotherapy. Notably, 10 (66.7 %) of the participants received concurrent systemic antimicrobials unrelated to CDAD. Clinical features of the presenting episode associated with subsequent RCDAD included presence of nausea or vomiting (100 % vs 27.3 % for RCDAD vs no RCDAD,  $P = 0.026$ ) and a lower peak white blood cell count ( $12.3 \times 10^3$  vs  $20.5 \times 10^3 \mu\text{l}^{-1}$ ,  $P = 0.025$ ). More participants with RCDAD received gastric acid suppression therapy after enrolment (100 % vs 54.5 % for RCDAD vs no RCDAD,  $P =$  not significant). There were no *Lactobacillus* infections, LGG-related serious adverse events or intolerances leading to study discontinuation. Mild to moderate adverse effects attributed to LGG included bloating (25 % incidence) and excessive flatulence (37.5 % incidence). Reported adherence to the assigned study capsules was  $\geq 90$  % for all but two study participants (one in each arm).

Evidence for efficacious treatments of RCDAD is sparse. Our study findings complement another small-scale trial that reported no benefit from a yogurt formulation of adjunctive LGG (Pochapin, 2000; McFarland, 2005). Although our study was not powered to detect a difference in outcomes for RCDAD, the data spotlight several feasibility challenges relevant to the

launch of a large-scale probiotic efficacy trial. First, the majority of participants were prescribed concurrent systemic antimicrobial therapy for non-CDAD indications and gastric acid suppressive therapy, both of which interfere with the normal gut flora and its ability to inhibit *C. difficile* colonization (McFarland, 2005; Dial *et al.*, 2004). Avoidance of these drugs is often not feasible in frequently hospitalized older adults with multiple comorbidities, thus introducing an important source of confounding that can be difficult to quantify. Second, 60-day retention in this study was arduous for many of the frail, elderly participants, and was often further complicated by concomitant cognitive dysfunction. Also, most study participants were presenting with a first recurrence of CDAD, unlike the patients with recalcitrant disease who were previously reported in case series of successful adjunctive LGG treatment (Gorbach *et al.*, 1987; Bennett *et al.*, 1996). Lastly, the participants were conservatively chosen to be at low risk for infectious complications from the study probiotic. Compared to previous RCDAD cohorts, the subsequent exclusion of younger immunocompromised patients resulted in an older study population with age-related gut flora changes possibly influencing the underlying risk for CDAD recurrence (McFarland *et al.*, 1994; Surawicz *et al.*, 2000; Wullt *et al.*, 2003; Hopkins & Macfarlane, 2002).

Together, these findings suggest that future probiotic studies should be designed to carefully measure and control for the receipt of confounding drugs that impact the gut flora, and should have end points that distinguish outcomes in time-to-event analyses. Careful consideration must also be made in choosing the study population to account for heterogeneous risk attributable to immune status, age, cognitive function and prior CDAD history. As emphasized by McFarland (2005), effective adjunctive treatments for preventing CDAD recurrence are lacking, and we echo the call for full-scale clinical trials to investigate those that appear promising.

**Table 1.** Characteristics and outcomes of RCDAD participants in adjunctive LGG probiotic trial

All comparisons between LGG and placebo arms not significant unless otherwise stated. SD, Standard deviation; WBC, white blood cell count.

Characteristic/outcome	No. (%) or mean (SD) or median (range)	
	LGG (n = 8)	Placebo (n = 7)
<b>Pre-enrolment and presenting-episode features</b>		
Mean age (years)	74.8 (14.4)	81.4 (5.3)
Female	8 (100.0)	5 (71.4)
Non-white	4 (50.0)	2 (28.6)
Participants presenting comorbidities		
Active malignancy	2 (25.0)	1 (14.3)
Diabetes mellitus	5 (62.5)	2 (28.6)
Dementia	4 (50.0)	1 (14.3)
Participants with prior CDAD episodes		
≥2 prior episodes	3 (37.5)	2 (28.6)
Early recurrence (<60 days since last episode)	4 (50.0)	6 (85.7)
Clinical features of presenting episode		
Median peak frequency of bowel movements (day <sup>-1</sup> )	8.0 (3–18)	8.0 (4–12)
10 <sup>-3</sup> × Mean peak WBC (μl <sup>-1</sup> )	13.7 (4.9)*	22.5 (4.5)*
<b>Post-enrolment</b>		
Recurrent CDAD as primary outcome	3 (37.5)	1 (14.3)
Median duration study capsules received (days)	24.5 (14–42)	35.0 (18–67)
Initial anti- <i>C. difficile</i> antibiotics for index episode		
Oral metronidazole only	7 (87.5)	5 (71.4)
Oral vancomycin only	0	1 (14.3)
Both metronidazole and vancomycin	1 (12.5)	1 (14.3)
Median duration of anti- <i>C. difficile</i> antibiotic regimen (days)	18.0 (16–27)	13.0 (10–21)
Participants receiving concurrent systemic antimicrobial agents	5 (62.5)	5 (71.4)
Participants receiving gastric acid suppression agents	6 (75.0)	4 (57.1)
Participants reporting symptoms related to study capsules†		
Nausea or vomiting	0	0
Abdominal pain	0	0
Constipation	0	0
Bloating	2 (25.0)	1 (14.3)
Excessive flatulence	3 (37.5)	0

\**P* = 0.005 by independent samples *t*-test. Data not available for one participant in the LGG arm.

†Probably related to study capsules; new onset or worsening of symptom post-enrolment.

## Acknowledgements

S. J. L. received salary support from National Research Service Award #5 T32 AI07 172-24. The probiotic and placebo capsules were donated by CAG Functional Foods, Omaha, Nebraska, USA. Potential conflict of interest:

J. R. K. has received research support from Rhodia Pharma Solutions; S. J. L and L. M. M., no conflict.

**Steven J. Lawrence,<sup>1</sup>  
Joshua R. Korzenik<sup>2</sup>  
and Linda M. Mundy<sup>3,4</sup>**

<sup>1</sup>Division of Infectious Diseases, Washington University School of Medicine, Box 8051, 660 South Euclid Avenue, St. Louis, MO 63110, USA

<sup>2</sup>Division of Gastroenterology, Harvard Medical School, Boston, MA, USA

<sup>3</sup>Philadelphia FIGHT, Philadelphia, PA, USA

<sup>4</sup>Saint Louis University School of Public Health, St. Louis, MO, USA

Correspondence: Steven J. Lawrence (slawrenc@im.wustl.edu)

**Bennett, R. G., Gorbach, S. L., Goldin, B., Chang, T.-W., Laughon, B. E., Greenough, W. B. & Bartlett, J. G. (1996).** Treatment of relapsing *Clostridium difficile* diarrhea with *Lactobacillus* GG. *Nutr Today* **31**, S35–S38.

**Dial, S., Alrasadi, K., Manoukian, C., Huang, A. & Menzies, D. (2004).** Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *CMAJ* **171**, 33–38.

**Gorbach, S. L., Chang, T.-W. & Goldin, B. (1987).** Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* **ii**, 1519.

**Hopkins, M. J. & Macfarlane, G. T. (2002).** Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *J Med Microbiol* **51**, 448–454.

**McFarland, L. V. (2005).** Alternative treatments for *Clostridium difficile* disease: what really works? *J Med Microbiol* **54**, 101–111.

**McFarland, L. V., Surawicz, C. M., Greenberg, R. N. & 10 other authors (1994).** A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA* **271**, 1913–1918.

**Pochapin, M. (2000).** The effect of probiotics on *Clostridium difficile* diarrhea. *Am J Gastroenterol* **95**, S11–S13.

**Surawicz, C. M., McFarland, L. V., Greenberg, R. N. & 8 other authors (2000).** The search for a better treatment for recurrent *Clostridium difficile* disease: use of high-dose vancomycin combined with *Saccharomyces boulardii*. *Clin Infect Dis* **31**, 1012–1017.

**Wullt, M., Johansson Hagslätt, M.-L. & Odenholt, I. (2003).** *Lactobacillus plantarum* 299v for the treatment of recurrent *Clostridium difficile*-associated diarrhoea: a double-blind, placebo-controlled trial. *Scand J Infect Dis* **35**, 365–367.