Published in final edited form as:

Anaerobe. 2016 October ; 41: 51–57. doi:10.1016/j.anaerobe.2016.05.007.

Probiotics as adjunctive therapy for preventing *Clostridium difficile* infection – What are we waiting for?

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Abstract

With the end of the golden era of antibiotic discovery, the emergence of a new post-antibiotic age threatens to thrust global health and modern medicine back to the pre-antibiotic era. Antibiotic overuse has resulted in the natural evolution and selection of multi-drug resistant bacteria. One major public health threat, *Clostridium difficile*, is now the single leading cause of hospital-acquired bacterial infections and is by far the most deadly enteric pathogen for the U.S. population. Due to the high morbidity and mortality and increasing incidence that coincides with antibiotic use, non-traditional therapeutics are ideal alternatives to current treatment methods and also provide an avenue towards prevention. Despite the need for alternative therapies to antibiotics and the safety of most probiotics on the market, researchers are inundated with regulatory issues that hinder the translational science required to push these therapies forward. This review discusses the regulatory challenges of probiotic research, expert opinion regarding the application of probiotics to *C. difficile* infection and the efficacy of probiotics in preventing this disease.

Keywords

Bio-K+; *Clostridium difficile*; fecal microbiota transplantation; Lifeway kefir; *Lactobacillus*; probiotic

1. Introduction

We are on the verge of a global antibacterial resistance crisis,^{1,2} where deaths due to common infections will rival heart disease and cancer and erase 70 years of progress combating life-threatening bacterial infections. This crisis was presaged by Sir Alexander Fleming in his 1945 Nobel Prize speech³ where he predicted that the misuse of antibiotics by the public at large would be the undoing of the “golden era” of antibiotics. A major risk

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factor of antibiotic therapy is acquiring *C. difficile* infection (CDI), with approximately half a million cases contributing to ~30,000 deaths per year in the United States at annual costs of \$4.8 billion.⁴ The majority of these cases occur following disruption of the gastrointestinal microbiota through antibiotic use (Figure 1) for non-related events. The current standard of care for CDI involves additional antibiotic therapy in the face of a disease already caused by previous antibiotic administration. *C. difficile* is not significantly resistant to drugs currently used to treat the disease; however some concerns about antibiotic resistance exist. Hypervirulent BI/NAP1/027 strains that emerged in the early 2000s are associated with fluoroquinolone resistance,⁵⁻⁹ and more recently the European EUCAST epidemiological marker of resistance for metronidazole and vancomycin has increased for *C. difficile* isolates in the U.S.¹⁰ Even more concerning is the prophylactic use of vancomycin or metronidazole to prevent CDI;^{11,12} oral administration of these drugs has been linked to a decline in intestinal microbiota colonization that imposes a greater risk for developing CDI.¹³ Despite a lack of significant antibiotic resistance by *C. difficile*, the unique relationship of CDI with antibiotic treatment and escalating incidence and mortality rates have driven the Centers for Disease Control and Prevention (CDC) to classify *C. difficile* as an urgent public health threat requiring urgent and aggressive action¹⁴.

A steady rise in CDI is accompanied by increased incidences of disease recurrence, an arguably larger problem with increased morbidity and mortality and fewer treatment options. Current treatment options for CDI are limited to extended antibiotic regimens with only three antibacterial drug choices and/or fecal microbiota transplantation (FMT). Most patients with CDI will respond to vancomycin or off-label use of metronidazole as the first line of treatment, although approximately 35% of patients will undergo a recurrence after antibiotic treatment.¹⁵⁻¹⁷ Almost 50% of these recurrent cases will face multiple recurrences, contributing further to the rise in patient morbidity.¹⁸ Severe recurrent cases are currently treated by FMT with 90% efficacy, demonstrating the efficacy of microbiota-based therapy. While FMT is an effective treatment option for patients with recurrent CDI,^{15,19} it is deemed an investigational therapy by the US Food and Drug Administration that may not be covered by third-party payers²⁰. Long-term health effects of FMT are still unknown; a recent case report highlighting new-onset obesity in a patient receiving stool from an overweight donor²¹ raises serious concerns about the lasting effects of FMT and emphasizes the importance of finding alternative treatment and prevention options for CDI. The ability to transfer obesity via FMT was first shown in animals.²² Similar animal studies are also demonstrating that other chronic conditions like high blood pressure, atherosclerosis, and potentially heart failure can be transferred via gastrointestinal microbiome transplantation.²³⁻²⁵ Probiotics have the most potential for use as adjunctive treatment to antibacterial therapy as a means to prevent primary and recurrent CDI. In spite of mounting literature reporting that probiotics are safe and effective for preventing CDI, clinicians are working from outdated 2010 SHEA/IDSA guidelines²⁶ that do not offer a consensus on the best treatment for recurrent CDI and deter use of probiotics for disease prevention.

The use of probiotics to treat gastrointestinal disease was well underway before Sir Alexander Fleming discovered the antagonistic effects of penicillin. The beneficial effects of bacteria were first described by Elie Metchnikoff, who touted that lactic acid bacteria could “replace the harmful microbes by useful microbes” in the intestine.²⁷ In 1917, Alfred Nissle

isolated the non-lactic acid *Escherichia coli* Nissle 1917 strain²⁸ which has since been used traditionally in Germany and other European countries to treat gastrointestinal afflictions.²⁹

More than a century later, instead of clinical use of defined microbes that are generally regarded as safe,³⁰ modern medicine is relying on 1,700 year old, non-standardized FMT practices.³¹ Several recent reviews^{32–39} and meta-analyses^{40–47} have covered the literature supporting probiotics as an alternative preventative method for CDI. For a summary of probiotic formulations tested in human trials for efficacy against CDI see Table 1. This review highlights the current opinion of experts in the field and the most recent clinical success stories that directly support continued effort towards developing alternative probiotic therapies for CDI prevention (Figure 1).

2. What the experts say

Human research on probiotics is significantly hindered by regulatory hurdles in the U.S. that hampers our ability to advance clinical applications of probiotics. Clear recommendations for clinical use of probiotics are lacking due to limited supporting evidence, which is more of an issue of regulatory hindrances than lack of efficacy of the probiotics themselves. Safety concerns have been raised in critically ill patient populations where the use of a probiotic has resulted in complications and even increased mortality.^{48,49} The intended use of a probiotic as a drug (defined by the Food and Drug Administration (FDA) as anything intended for diagnosis, cure, mitigation, treatment, or prevention of disease)⁵⁰ subjects the probiotic to FDA regulations requiring an Investigational New Drug (IND) application for human research examining health benefits. An IND is required despite a probiotic being available for public consumption and generally regarded as safe³⁰ (Figure 2). The requirement of an IND to conduct the necessary clinical research to attach health claims to probiotics already deemed safe for general public consumption precludes scientists from pursuing such studies.⁵¹ The explosion of human microbiome research has increased the public's interest in beneficial microbes and intensified the pressure on physicians to make recommendations for clinical probiotic use. In the face of growing public interest, and despite outdated SHEA/IDSA guidelines²⁶ deterring the clinical use of probiotics in CDI due to “insufficient evidence”, clinicians are making general recommendations to patients on antibiotic therapy.^{52–54} The World Gastroenterology Organization Global Guidelines⁵⁵ concur that there is strong evidence showing the efficacy of specific probiotics at preventing CDI, but agree with the FDA and others that more evidence based on randomized, placebo-controlled, blinded studies is required before specific products can be ranked with proven efficacy.

Extensive use of probiotics by physicians and patients in the absence of clear recommendations^{52–54,56} was the basis for founding the Yale University Workshop on Probiotic Recommendations that assesses the scientific literature regarding the efficacy of probiotics. This panel of probiotic experts consistently reports that the literature substantiates the use of probiotics in the case of antibiotic-associated diarrhea.^{57–60} Recommendations on the use of probiotics in CDI were first made at this workshop in 2008,⁵⁸ concluding that there were positive, controlled studies (with the presence of some negative data) supporting the use of probiotics as adjuvant or preventative therapy to CDI in

adults. The recommendation from this group that the use of probiotics in CDI is supported by the literature has continued, although they highlight more data are needed to support clear recommendations regarding specific dosages and strains.^{38,61} These recommendations were further substantiated by an expert panel of Canadian and U.S. infectious disease specialists that specifically addressed CDI prevention strategies and included the use of adjuvant therapy with specific probiotics (Bio-K+⁶²) in their recommendations.³⁷ However, in the case of pediatrics the literature is scarce³³ and the Yale Workshop deemed that there is a “lack of evidence strong enough to build any recommendation about ‘routine’ use,”³⁸ concurrent with the American Academy of Pediatrics which does not include a recommendation on probiotics for CDI.⁶³

3. Recent significant clinical probiotic successes

Recommendations made by the Yale Workshop do not include data from two key probiotic trials highlighted below that utilized probiotics as adjunctive therapy to antibiotics. While neither study was a randomized, placebo controlled trial, both administered a probiotic product containing multiple bacterial strains (Lifeway® kefir or Bio-K+) to patients receiving antibiotics under severe circumstances. Results from these studies, which reported no negative outcomes, strongly suggest that probiotic-based adjunctive therapy reduces primary and recurrent CDI incidence. Additionally, these studies were conducted in predominantly aging populations known to be at high risk for developing CDI.

3.1 Lifeway® kefir shows promise for recurrent CDI prevention in elderly patients

Patients >65 years of age most at risk for developing recurrent CDI may not be able to undergo FMT for various reasons, including cost or the presence of co-morbidities. Due to the requirement for a non-invasive, cost-effective alternative to FMT, the efficacy of a probiotic product as adjunctive therapy for preventing recurrent CDI in this patient population was assessed. Twenty-five patients averaging 68 years of age with severe, recurrent CDI and varying comorbidities were given a choice between (1) FMT or (2) staggered and tapered antibiotic withdrawal (STAW) therapy supplemented with Lifeway® kefir,^{64,65} a fermented milk product that contains 7–10 billion colony forming units of 12 viable probiotic organisms (www.kefir.com), many of which are members of species known to be intrinsically resistant to antimicrobials used to treat recurrent CDI. The STAW therapy, chosen by all study participants, entailed six weeks of staggered and tapered metronidazole or vancomycin supplemented with a minimum of three 5-oz Lifeway® kefir drinks daily at the onset of STAW therapy and for two months after STAW completion. Antibiotic dosage occurred every 72 hours with a gradual reduction every two weeks for the six week period. This study reported complete resolution of symptoms in 84% of patients using this treatment regimen following a recurrent CDI diagnosis. Comorbidities were not associated with the four cases of treatment failure and no complications were reported.

Extended antibiotic therapy supplemented with a cocktail of beneficial microbes may therefore constitute an effective means of preventing recurrent CDI in an elderly population with varying comorbidities. While these results are promising, confirmation in a multi-site cohort are lacking and attempts to reproduce the effects of Lifeway kefir in a mouse model

of CDI have demonstrated limited efficacy,⁶⁶ highlighting the difficulties of studying translational human therapies in animal models. Repeated multi-center clinical trials using kefir with larger patient populations are needed to solidify kefir supplementation as a viable adjunctive therapy for prevention of recurrent CDI.

3.2 Bio-K+ maintains CDI prevention and reduces severity for a decade

In 2003, hospitals in Montreal, Canada experienced a spike in CDI incidence from 5.7 to 28.2 cases per 1,000 patient admissions due to the emergence of the hypervirulent, fluoroquinolone-resistant *C. difficile* strain BI/NAP1/027^{9,67} responsible for 50–70% of isolates in the Quebec hospital network.⁶⁸ The increased disease severity and mortality associated with this outbreak prompted Pierre-Le Gardeur Hospital (PLGH) in Quebec, Canada to mandate an adjunctive probiotic intervention to all adult inpatients receiving antibiotics.^{68,69} PLGH initially responded to this outbreak by augmenting standard protective measures (aSPM) and increasing antibiotic stewardship. The aSPM were ineffective at decreasing CDI rates; in fact CDI incidence increased during this six month period. As a result, the Infection Prevention Committee mandated the use of a probiotic as an adjunctive treatment for all patients over the age of 18 receiving antibiotics at PLGH; no adult patients were excluded from the treatment plan. The probiotic of choice, Bio-K+ (https://www.biokplus.com/en_ca) is a three-strain cocktail that was given within 2–12 hours of starting antibiotics for a minimum of 30 days or until antibiotic treatment was completed. Daily dosage of Bio-K+ was age dependent with patients 18–49 years old receiving 50 billion CFU and patients 50 years old receiving 100 billion CFU.

Over the course of ten years, 44,835 inpatients (99.5% compliance) received antibiotics supplemented with Bio-K+⁶⁸ at PLGH. CDI incidence plummeted from 18.0 to 2.3 cases per 10,000 patient-days, and PLGH consistently had lower CDI rates than 95 other hospitals in the Quebec region.⁶⁸ Recurrence rates decreased 39% in the first 18 months, and no severe multiple recurrent nosocomial cases (>2 recurrences after adequate treatment) requiring FMT have been reported at PLGH since implementing Bio-K+ adjunctive therapy. Additionally, *Lactobacillus* spp. were never identified as the causative agent of bacteremia during a decade-long surveillance of blood cultures at PLGH. Ultimately, no treatment-related adverse events due to Bio-K+ were reported. The results of this study led Maziade, *et al*^{68,69} to conclude that Bio-K+ is extremely safe and highly effective at preventing CDI and reducing disease severity when administered concurrently with antimicrobials. This probiotic formulation is included in recent recommendations by Goldstein, *et al*.³⁷

4. Where do we go from here?

The cumulative clinical data continues to lean towards supporting probiotics as adjunctive therapy for prevention of CDI. However, several points should be emphasized as we progress forward. For instance, there is no generic equivalency between probiotics – not any bug will do. Recommendations should be made on a strain or formulation specific basis substantiated by scientific evidence based on clinical trials. This point is supported by the differing results obtained from studies assessing probiotics in patients >65 years of age. The two studies highlighted in this review^{64,65,68,69} clearly indicate that specific probiotic

formulations (Lifeway kefir and Bio-K+) and dosages are effective at preventing both primary and recurrent CDI in older, high-risk patients when administered with antibiotic therapy. However, two clinical trials using different formulations of multi-strain probiotics^{70,71} showed little efficacy in elderly populations, resulting in the general conclusion that “probiotics” are not effective in patients >65 years of age. Further support for recommending specific strains or formulations is garnered by clinical trials testing the effectiveness of *Saccharomyces boulardii* at preventing CDI when provided with antibiotics. Earlier studies indicate that *S. boulardii* is effective at preventing recurrent CDI,^{61,72} while more recent studies have indicated that *S. boulardii* is not effective at preventing CDI.^{73,74} Strain designations were not detailed in these publications further contributing to the overgeneralization of the effects (or lack thereof) of a probiotic species as a whole.

Microbiome studies^{75–79} indicate that relative abundances of a variety of *Lactobacillus* species are maintained during antibiotic treatment. Many probiotic *Lactobacillus* species are intrinsically resistant to the drugs used to treat CDI⁸⁰ and are therefore well suited to withstand the current CDI treatment standards as adjunctive therapies. Supplementation of specific *Lactobacillus* organisms and other naturally resistant microbes may preserve host defenses in the fight against antibiotic-associated CDI. Emerging data suggests that specific microbiome perturbations resulting from different CDI treatment regimens may be predictive of treatment outcomes.^{78,81} It will be important to determine if these same treatment-associated perturbations impact the benefits of probiotics.

The use of safe and defined probiotic formulations as microbiota replacement therapy with specific beneficial properties that ward off pathogens and enhance human health (Figure 2) would be a safer, long-term solution to CDI prevention. It remains to be seen if probiotic prevention strategies for CDI should include single- or multi-strain formulations. Specific strains with defined beneficial properties relevant to combating virulence and enhancing host defenses specific to CDI are being targeted. A recent clinical trial using the single organism, *L. plantarum* 299v (10 billion CFU daily), prophylactically in a high-risk patient population taking antibiotics showed a significant decrease in CDI incidence (0.11% vs 1.21%; $p = 0.0001$).⁸² Studies indicating the importance of bile acid metabolism by GI microbiota in providing moderate *C. difficile* colonization resistance in a mouse model^{81,83} suggest that single organisms with specific functions may be sufficient. However, multi-strain bacteriotherapies may be more akin to FMT at modulating microbial communities and providing increased diversity after sequential antibiotic treatments. The studies highlighted in this review employed probiotic formulations containing 12 (Lifeway kefir) or 3 (Bio-K+) defined organisms^{64,65,68,69} (Table 1). Moreover, microbial concoctions from healthy donor stool have shown efficacy against CDI in animal models^{81,84} and human clinical trials.^{85–87} Ultimately, targeted delivery systems containing a combination of prebiotic and probiotic formulations could provide a more efficient means of fighting CDI by directly influencing *C. difficile* pathogenicity and the microbiota via modulation of the metabolome. CDI-associated gut metabolites are reviewed further by Ross, *et al*⁸⁸ in this special issue.

While the application of probiotics to CDI treatment and prevention has been controversial, a consensus is emerging that probiotics have strong potential as adjunctive therapies to antibiotics. Experts contend that continued effort in determining specific probiotic

formulations and doses through well-conducted human trials are required to solidify clear recommendations for probiotic applications in health care. Defined microbial supplements show promise not only in preventing primary CDI, but also in severe cases of recurrence; Canadian and U.S. experts are recommending Bio-K+ be bundled with other preventative measures to control CDI.³⁷ During this antimicrobial resistance crisis there is an urgent need for alternative treatment strategies to prevent all forms of CDI. Current treatment guidelines for CDI express that there is insufficient evidence to conclusively recommend probiotics as safe preventative strategies, while U.S. regulatory obstacles hinder scientists' ability to conduct the clinical trials required to produce sufficient evidence for making said recommendations. There is no question that more must be done. With the CDC sounding the alarm against antimicrobial resistance and the FDA currently reviewing their policy for human research on foods, there is hope that changes will enable probiotic research to progress in the U.S.

Acknowledgments

Karen Prince's assistance in preparing figures is appreciated. This work was supported by grants RO1AI100914, DK096323 and P30DK56338 from the National Institute of Allergy and Infectious Diseases and National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health; by the Pediatric GI Training Grant T32-DK07664; and by the Department of Pathology, Texas Children's Hospital.

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Clostridium difficile disease progression

Probiotic Prevention Strategy

Figure 1.

Disruption of a healthy GI microbiota by antibiotics increases CDI susceptibility. Adjuvant probiotic therapy provides protection against CDI by stabilizing the GI microbiota, protecting the host by various mechanisms and preventing *C. difficile* invasion.

Figure 2.

An illustration of the discordance in regulations for probiotic foods. Probiotics, generally regarded as safe (GRAS), may be consumed by the general public without clear recommendations for specific applications. Attempts to translate probiotic research through human trials on the same GRAS organisms are hindered by the Food and Drug Administration's (FDA) requirement for an Investigational New Drug (IND) application, decreasing the necessary clinical trial data required for physicians' to make clear recommendations on the clinical use of probiotics.

Table 1

Summary of evidence-based indications of probiotic use in CDI.

Probiotic Organism(s) (Marketed Product)	Dose (CFU/day)*	Study Population	Country	CDI Study Aim	Reference
Single Strain Formulations					
<i>L. plantarum</i> 299v (Sanprobi IBS®)	10×10 ⁹	Adult	Sweden	Prevention	Lonnermark, 2010 ⁸⁹
	10×10 ⁹	Elderly	Poland	Prevention	Kujawa-Szewieczek, 2015 ⁸²
<i>L. rhamnosus</i> GG	2×10 ¹⁰ , twice daily	Pediatric	Finland	Prevention	Arvola, 1999
	2×10 ¹⁰	Pediatric	Poland	Prevention	Ruszczynski, 2008 ⁹⁰
<i>S. boulardii</i>	10×10 ⁹	Pediatric	Poland	Prevention	Kotowska, 2005 ⁷²
	30×10 ⁹	Adult	USA	Treatment/Prevention of Recurrent CDI	McFarland, 1994, ⁹¹
	20×10 ⁹	Adult	USA	Treatment/Prevention of Recurrent CDI	Surawicz, 2000 ⁹²
Multi-Strain Formulations					
<i>L. acidophilus</i> <i>B. bifidum</i> (Cultech, Swansea)	20×10 ⁹	Elderly	England	Prevention	Plummer, 2004 ⁹³
<i>L. acidophilus</i> CL1285® <i>L. casei</i> LBC80R® <i>L. rhamnosus</i> CLR2® (Bio-K+®)	25–50×10 ⁹	Adult	Canada	Prevention	Beausoleil, 2007 ⁹⁴
	25–50×10 ⁹	Adult	Canada	Prevention	Sampalis, 2010 ⁹⁵
	50–100×10 ⁹	Adult	China	Prevention	Gao, 2010 ⁹⁶
	25–50×10 ⁹	Adult and Elderly	Canada	Prevention	Maziade, 2013 ⁶⁹ Maziade, 2015 ⁶⁸
<i>L. casei</i> imunitass DN-114 <i>L. bulgaris</i> <i>S. thermophilus</i> (Actimel)	19×10 ⁹	Adult	England	Prevention	Hickson, 2007 ⁷¹
<i>B. breve</i> <i>B. lonum</i> <i>B. infantis</i> <i>L. acidophilus</i> <i>L. plantarum</i> <i>L. paracasei</i> <i>L. bulgaricus</i> <i>S. thermophilus</i> (VSL#3)	900×10 ⁹	Adult	UK	Prevention	Selinger, 2013 ⁹⁷
<i>B. breve</i> <i>B. lactis</i> <i>B. longum</i> <i>L. acidophilus</i>	3× 5-oz/day	Elderly	USA	Treatment/Prevention of Recurrent CDI	Bakken, 2009, ⁶⁴ Bakken, 2014 ⁶⁵

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Probiotic Organism(s) (Marketed Product)	Dose (CFU/day)*	Study Population	Country	CDI Study Aim	Reference
<i>L. casei</i>					
<i>L. lactis</i>					
<i>L. plantarum</i>					
<i>L. reuteri</i>					
<i>L. thamnosus</i>					
<i>Leuconostoc cremoris</i>					
<i>S. florentinus</i>					
<i>S. diacetylactis</i>					
(Lifeway® Kefir)					

* For complete dosage details, see published study referenced.