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Is primary prevention of *Clostridium difficile* infection possible with specific probiotics?

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SUMMARY

Background: The efficacy of probiotics for the prevention of *Clostridium difficile* infection (CDI) is highly controversial, particularly with regard to the prevention of recurrent CDI. We hypothesize that primary prevention of CDI among patients receiving antibiotics might be a more achievable goal for probiotics than prevention in patients with previous CDI where the host flora is markedly altered.
Methods: We conducted a literature search for randomized, placebo-controlled efficacy studies of probiotic use among adults receiving antibiotics, in which CDI was one of the outcomes measured. In addition, we conducted meta-analyses of probiotics that were included in more than one randomized trial.
Results: Eleven studies were identified; most were seriously underpowered to determine the efficacy of probiotics in the prevention of CDI. Two showed significantly lower rates of CDI among the probiotic recipients. A meta-analysis of three studies that used the probiotic combination *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R and a combined analysis of those studies with four studies that used *Saccharomyces boulardii*, showed lower CDI rates in recipients of probiotics compared with recipients of placebo (risk ratio = 0.39; 95% confidence interval 0.19–0.79).
Conclusions: While potential flaws in study design were identified, a review of the available literature suggests that the primary prevention of CDI with specific probiotic agents may be achievable. Additional studies of sufficient size and with rigorous design are needed to confirm these findings.
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1. Introduction

Antibiotics are the major risk factor for a primary episode of *Clostridium difficile* infection (CDI), as well as an important factor for recurrent CDI. The risk associated with antibiotics primarily relates to disruption of the protective host colonic microbiota, but may also involve selection for *C. difficile* strains resistant to the inciting agent.[1] Adjunctive therapy with probiotics has been used widely for patients with CDI, with and without the guidance of physicians. The goal of probiotic therapy is to mitigate the effects of microbiota disruption, and different mechanisms have been proposed whereby specific probiotics affect the microbiota and interfere with *C. difficile*.[2]

Most studies of CDI prevention have focused on secondary prevention (i.e., prevention of CDI recurrence), mainly because the risk for CDI is sufficiently high in patients with a recent CDI episode that the effect of intervention is easier to demonstrate; 20–30% after the first episode and ~50% after the second episode.[3] The rate of primary episode CDI among antibiotic recipients varies with different antibiotics and populations studied, but is much lower than the rate of recurrent CDI and usually is much less than 10%.[4] Therefore, a larger study population is needed to demonstrate efficacy in primary CDI prevention.

The efficacy of probiotics in the prevention of CDI has been hotly debated,[5,6] but many studies and meta-analyses have combined primary and secondary CDI prevention data, which

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potentially obscures important differences in the effectiveness of probiotic interventions between these populations. It is our hypothesis that probiotics may be more effective in primary CDI prevention (those patients at risk for CDI) than in secondary prevention of recurrent CDI in patients with an established *C. difficile* infection. This study includes a general overview of probiotics studied for CDI prevention and a systematic analysis of randomized studies of primary prevention of CDI with probiotics.

1.1. Probiotics studied for the prevention of CDI

1.1.1. Specific probiotic organisms

Several bacterial and fungal species have been studied or are under study to determine their efficacy against CDI either as single probiotic agents or in combination with other agents (Table 1). These agents include *Saccharomyces boulardii* and several *Lactobacillus*, *Clostridium*, *Streptococcus*, and *Bifidobacterium* species. *S. boulardii* and *Lactobacillus rhamnosus* GG are two of the best characterized probiotic organisms for use in CDI.

S. boulardii is a yeast that is a subspecies of *Saccharomyces cerevisiae*, which was initially isolated from lychee and mangosteen fruits. It secretes a 54-kDa protease, in vivo.[7] In vitro, this protease has been shown to both degrade toxins A and B secreted from *C. difficile* and inhibit their binding to receptors along the brush border, leading to a reduction in the enterotoxic and cytotoxic effects of *C. difficile*.[7] *L. rhamnosus* GG is a naturally-occurring strain that was isolated from human feces and was selected for its potential probiotic properties: acid- and bile-stability, great avidity for human intestinal mucosal cells, reduction of intestinal permeability defects, and enhancement of intestinal immunity.[2]

Bio-K+ CL1285 is a probiotic combination (*Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R) that has been studied in three recent clinical trials of antibiotic-associated diarrhea (AAD) and CDI,[8–10] and has been shown to survive acid, pepsin, and bile salt in vitro.[11]

1.1.2. Probiotic formulations and correlates of efficacy

Probiotics, in general, vary widely in their manufacturing processes, and many commercial products lack regulated quality control programs resulting in contamination with other organisms, as well as bacteria counts that are different from the label on the product.[6] Stability of the product and viability of the organism at the time of ingestion are critical factors for efficacy and are not always guaranteed by the manufacturer. In contrast, *S. boulardii* and Bio-K+ CL1285 have undergone rigorous testing to ensure organism viability and concentration.[5] Bio-K+ CL1285 has been formulated as fermented milk[8,9] and freeze-dried bacteria placed in an enteric coated capsule.[10] Both products contain 50 billion live and active *L. acidophilus* CL1285 and *L. casei* LBC80R at the date of consumption (140 days for fermented milk and 2 years for capsules if kept refrigerated). Validation of the probiotic concentration in samples of Florastor (*S. boulardii*) and Bio-K+ obtained from the store was recently reported.[12] Susceptibility of the probiotic organisms to particular antibiotics is also theoretically important, as these products are usually recommended to be given during or shortly after antibiotic administration. However data on susceptibility or relative susceptibility of probiotics to the multiple different antibiotics that a patient may receive are scarce.

Table 1
Probiotics studied for prevention of *Clostridium difficile* infection

Single agent formulations
Saccharomyces boulardii
Lactobacillus rhamnosus GG
Lactobacillus plantarum 299v
Clostridium butyricum M588
Clostridium difficile VP20621 (non-toxigenic *C. difficile* strain)
Combination agent formulations
Lactobacillus casei, *Lactobacillus acidophilus* (Bio-K+CL1285)
Lactobacillus casei, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* (Actimel)
Lactobacillus acidophilus, *Bifidobacterium bifidum* (Florajen3)
Lactobacillus acidophilus, *Lactobacillus bulgaricus delbrueckii* subsp. *bulgaricus*, *Lactobacillus bulgaricus casei*, *Lactobacillus bulgaricus plantarum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Streptococcus salivarius* subsp. *thermophilus* (VSL#3)

Another factor that appears to be critical for probiotic efficacy is the number of organisms given. In one review of 25 randomized trials, administration of probiotics at concentrations of $\geq 10^{10}$ colony forming units (CFU)/day was associated with efficacy.[5] Both *S. boulardii* and Lactobacillus GG (LGG) can be detected in stool samples of patients ingesting these probiotics. *S. boulardii* is cleared within 3 days after stopping dosing,[13] but LGG may be detected for up to 7 days in ~30% of subjects.[2] The fecal concentration of *S. boulardii* also predicts recurrence of CDI. When given for 28 days after standard antibiotic treatment for CDI, patients with low *S. boulardii* concentrations ($<10^4$/g stool) experienced recurrences more often than those with levels $>10^4$/g.[13]

1.2. Animal models of CDI prevention

Animal models provide a useful method to evaluate the effectiveness of probiotics for the prevention of CDI. In hamsters pre-treated with antibiotics, the administration of cecal homogenates containing the complete indigenous microbiota were highly effective in providing protection from the development of cecitis after challenge with toxin-producing *C. difficile*.[14] These data are consistent with the subsequent demonstration that stool transplantation is effective in preventing recurrences of CDI in patients.[15] Specific non-toxigenic strains of *C. difficile* have been shown to effectively colonize clindamycin-treated hamsters and offer at least 95% protection against subsequent lethal challenge with virulent, toxin-producing strains.[16] In hamsters treated for CDI with vancomycin, *S. boulardii* treatment resulted in a modest reduction in *C. difficile* counts and a more dramatic decrease in toxin levels (3% of *S. boulardii*-treated hamsters had detectable toxin B versus 51% of controls).[17] In mice, *S. boulardii* did not reduce the concentration of *C. difficile*, but markedly reduced levels of toxins A and B and prevented damage to the intestinal mucosa.[18] Two mouse model studies have suggested a potential role for lactobacilli in reducing the severity of CDI,[19,20] but the conclusions that can be drawn from these small-scale studies are limited. There is a need for additional animal model studies to evaluate the effectiveness of various probiotic preparations for the prevention of CDI.

1.3. Potential risks of probiotics

The primary concern for adverse outcomes associated with probiotic use is the acquisition of probiotic-related infections and their associated complications. In the USA, probiotics have been regulated as dietary supplements by the Food and Drug Administration (FDA);[21] as a consequence, probiotics have never been subjected to the comprehensive safety evaluations that pharmaceuticals receive, and robust probiotic safety data are scarce. However, the FDA established a requirement for reporting probiotic adverse events in 2006.[21]

Population-based studies have found no increase in the incidence of Lactobacillus bacteremia after the introduction and widespread use of *Lactobacillus paracasei*, *L. acidophilus*, and *L. rhamnosus* probiotics in dairy products either in Finland or

Table 2
Randomized controlled trials of probiotics for primary CDI prevention in patients receiving antibiotics

Probiotic (daily dose, CFU)	Population (*n*, number evaluated)	Treatment duration (follow-up duration)	CDI rate		*p*-Value	Power to detect $p \leq 0.05$	Comments	Reference
			Placebo	Probiotic				
Saccharomyces boulardii 2×10^{10}	Adult inpatients (180)	Duration of antibiotics + 14 days (no f/up)	5/64 (7.8%)	3/116 (2.6%)	NS	26.5%	1 USA hospital	Surawicz et al., 1989[33]
S. boulardii 3×10^{10}	Adult inpatients (193)	Duration of antibiotics + 3 days (7 weeks f/up)	4/96 (4.2%)	3/97 (3.1%)	NS	2.6%	4 USA hospitals, patients on beta-lactam antibiotics	McFarland et al., 1995[34]
S. boulardii 4.5×10^{9}	Elderly patients[a] (69)	Duration of antibiotics (no f/up)	3/36 (8.3%)	5/33 (15.2%)	NS	7.2%	1 UK hospital	Lewis et al., 1998[35]
S. boulardii 1×10^{10}	Adult inpatients (151)	Duration of antibiotics (4 weeks f/up)	2/78 (2.6%)	0/73 (0%)	NS	9.1%	1 Turkish hospital	Can et al., 2006[36]
Lactobacillus acidophilus, *Bifidobacterium bifidum* 2×10^{10} CFU	Elderly adults[a] (138)	20 days (no f/up)	5/69 (7.2%)	2/69 (2.9%)	NS	11.5%	1 UK hospital	Plummer et al., 2004[39]
Lactobacillus rhamnosus GG 2×10^{10} CFU	Adult inpatients (267)	14 days (7 days f/up)	3/134 (2.2%)	2/133 (1.5%)	NS	2.7%	1 USA hospital	Thomas et al., 2001[40]
Lactobacillus casei, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* 4.2×10^{10} CFU	Adults (112)	Duration of antibiotics + 7 days (f/up 4 weeks after discharge)	9/53 (17.0%)	0/56 (0%)	$p = 0.001$	80.8%	3 UK hospitals, patients on high risk antibiotics excluded[b] (Actimel)	Hickson et al., 2007[41]
L. acidophilus, *L. bulgaricus*, *B. bifidum*, *S. thermophilus* 1.5×10^{9} CFU each or total of 6×10^{9}/day	Adult inpatients (42)	3 weeks (no f/up)	1/21 (4.8%)	3/21 (14.3%)	NS	7.2%	1 Israeli hospital (BioGuard)	Stein et al., 2007[42]
L. acidophilus CL1285, *L. casei* LBC80R 5×10^{10} CFU	Adult inpatients (89)	Duration of antibiotics (additional 21 days f/up)	7/45 (15.6%)	1/44 (2.3%)	NS ($p = 0.06$)	44.2%	1 Canadian hospital, 9-month study (Bio-K+, fermented milk)	Beausoleil et al., 2007[8]
L. acidophilus CL1285, *L. casei* LBC80R 5×10^{10} CFU	Adults (437)	Duration of antibiotics + 5 days (additional 21 days f/up)	4/221 (1.8%)	1/216 (0.5%)	NS	12.5%	8 Canadian hospitals, patients on antibiotics for 3–14 days (Bio-K+, fermented milk)	Psaradellis et al., 2010[9]
L. acidophilus CL1285, *L. casei* LBC80R 5×10^{10} CFU or 10×10^{10} CFU	Adult inpatients (255)	Duration of antibiotics + 5 days (additional 21 days f/up)	20/84 (23.8%)	Low dose: 8/85 (9.4%) High dose: 1/86 (1.2%)	Low dose: $p = 0.03$ High dose: $p = 0.002$	Low dose 64.0% High dose 99.2%	1 Chinese hospital, 3-month study (Bio-K+, capsules)	Gao et al., 2010[10]

CDI, *Clostridium difficile* infection; CFU, colony forming units; NS, not significant.
[a] Elderly was defined as age >65 years,[35] or as participants enrolled at a hospital for the elderly.[39]
[b] High risk antibiotics were defined as clindamycin, cephalosporins, and aminopenicillins.[41]

Sweden.[22,23] However, sporadic case reports of probiotic-related infections with both *S. boulardii*- and *Lactobacillus* species-based probiotic preparations have occurred,[24–29] primarily in immunocompromised patients, such as those with severe neutropenia, HIV, and malignancy.[24–26]

To date, the most rigorous probiotic safety data have been generated for two Lactobacillus combination preparations. A study investigating 1176 inpatients who were administered an *L. acidophilus* and *Lactobacillus bulgaricas* combination estimated a maximal probiotic-related bacteremia rate of 0.2%.[30] The patient population was well characterized and 47% had moderate or severe immunosuppression, 33% had impaired intestinal integrity, 20% had abnormal heart valves, and 54% were prescribed concurrent proton pump inhibitors. Only two suspected *L. acidophilus* bacteremias were identified, and neither infection could be definitively determined as probiotic-related as patient isolates were unavailable for molecular typing and comparison to probiotic strains.

Similarly, an *L. acidophilus* CL1285 and *L. casei* LBC80R combination has been administered to 781 adult inpatients in three trials,[8–10] without a single probiotic infection. However these trials specifically excluded immunosuppressed patients. More compelling is the report of the administration of this combination product to 30 000 adult inpatients receiving antibiotics at a single hospital, without any patient exclusions and without a single recognized episode of probiotic-related bacteremia.[31]

2. Methods

2.1. Analysis of randomized primary CDI prevention studies—systematic review search strategy

PubMed, Medline, and Google Scholar were searched for the period 1976 to 2010 for articles unrestricted by language. Three on-line clinical trial registers were searched: Cochrane Central Register of Controlled Trials (http://www.cochrane.org), *meta*Register of Controlled Trials (www.controlled-trials.com/mrct), and the National Institutes of Health (www.clinicaltrials.gov). Secondary and hand searches of reference lists, other studies cross-indexed by authors, reviews, commentaries, books, and meeting abstracts were also performed. Search terms included: *Clostridium difficile* disease, probiotics, Lactobacillus, BioK+, LGG, *Saccharomyces boulardii*, yeast, diarrhea, randomized controlled trials, placebo-controlled, phase 3, and associated author names. Search strategies were broad-based initially, then narrowed to the disease of interest to increase the search network.[32]

The main objective of this literature search was to determine the overall efficacy of probiotics for the primary prevention of CDI by comparing a common outcome in treated patients with a control group. Inclusion criteria included: randomized, controlled, blinded efficacy trials of primary prevention in adult patients published as full articles and where CDI was one of the outcomes measured.

2.2. Meta-analysis of probiotic primary CDI prevention studies

We performed a meta-analysis to explore the between-trial heterogeneity for specific probiotic formulations and to provide more precise estimates of formula-specific effect sizes. As such, and to minimize the noise from formulations that only had a single randomized controlled trial, we conducted a meta-analysis on trials involving probiotic agents that were studied in more than one trial. Two probiotic preparations have been studied in more than one randomized trial: *L. acidophilus* + *L. casei* (Bio-K+ International, Laval, QC; CL1285) ($n = 3$) and *S. boulardii* ($n = 4$). We analyzed data from the published randomized controlled trials in which patients receiving antibiotics were randomized to either *L. acidophilus* + *L. casei* or placebo,[8–10] and *S. boulardii* or placebo,[33–36] and in which AAD and CDI were the primary and secondary outcomes, respectively. We analyzed only the CDI outcome. All the *L. acidophilus* + *L. casei* studies used a fermented milk or capsule formulation of 5×10^{10} CFU *L. acidophilus* CL1285 and *L. casei* LBC80R. One study had an additional 'high dose' arm in which two doses were given (high dose 10×10^{10} CFU);[10] for this study, we aggregated results for the high- and low-dose formulations and compared them to placebo. The *S. boulardii* studies used capsule formulations containing between 4.5×10^9 and 3×10^{10} CFU of *S. boulardii*. We expressed the efficacy of *L. acidophilus* + *L. casei* or *S. boulardii* on the incidence of CDI as relative risks (values <1 indicate a benefit) with 95% confidence intervals (95% CI). We present the results for each probiotic preparation separately and the overall effect. We used the metan command for Stata,[37] using Stata version 10.1 software (Stata Inc., College Station, TX, USA). We specified a random effects analysis. We report the I^2 result as a measure of inter-study heterogeneity.[38]

3. Results

3.1. Description of randomized primary CDI prevention trials

We identified 11 published studies[8–10,33–36,39–42] among adults receiving antibiotics who were randomized to a probiotic or probiotic combinations versus placebo and in which CDI was one of the outcomes measured (Table 2). The primary outcome measure in the majority of these studies was AAD, and CDI was a secondary outcome. Four of these studies involved *S. boulardii* and seven involved *Lactobacillus sp* alone ($n = 1$) or in combination with other *Lactobacillus sp* or other organisms ($n = 6$). The population studied was hospitalized patients in almost all cases, and the studies were conducted in the USA (three studies), UK (three studies), Canada (two studies), and Israel, Turkey, and China (one study each). The rate of CDI among the placebo groups was less than 10% for eight of the studies. Most studies were not powered sufficiently to detect a significant difference; only two studies had >60% power to detect a significant difference of $p < 0.05$ (Table 2). There were more CDI cases in the placebo group than in the probiotic group in nine of the 11 studies, but the difference was only significant in two studies.[10,41] The rates of CDI in the placebo recipients in these two studies (17.0% and 23.8%) were the highest among all the studies, and the study with the next highest CDI rate in placebo recipients (15.6%) showed a strong trend towards probiotic effectiveness ($p = 0.06$).[8] This latter study was conducted between September 2003 and May 2004 in a Montreal hospital during a multi-hospital CDI outbreak in the Montreal area;[43] this timing may explain the high rate of CDI among placebo recipients. It is unknown if there was an outbreak at the time of the studies in the UK and Chinese hospitals at which the placebo CDI rates were the highest.[10,41] The epidemiology of CDI in China, in particular, is unclear, and the Chinese study was conducted at a single Shanghai hospital where 255 patients were enrolled over a 3-month period in 2009.[10]

There are caveats to keep in mind in the comparison of these reports, as there were several differences in study design, including population studied, probiotic treatment duration, length of follow-up (Table 2), exclusion criteria, and non-systematic testing of patients with AAD for *C. difficile*. The more recent studies explicitly mentioned prior CDI as an exclusion criterion[8–10,40] or screened patient stools at baseline for *C. difficile*.[39,41,42] The *S. boulardii* studies, however, did not specifically mention CDI as an exclusion criterion.[33–36] Although patients with prior CDI could potentially have been enrolled in these studies, all of the studies excluded patients with recent diarrhea episodes and two excluded patients

receiving treatment agents for CDI (e.g., oral vancomycin, teicoplanin, or metronidazole).[33,36]

3.2. Meta-analysis of multiple trials employing the same probiotic combinations

The three most recently reported studies used the same probiotic, a formulation of 5×10^{10} CFU *L. acidophilus* CL1285 and *L. casei* LBC80R (Bio-K+ International, Laval, QC, Canada), and were conducted using similar methods (Table 2). A meta-analysis of these studies showed an overall effect of lower CDI rates among those taking the probiotic compared with those taking the placebo (risk ratio 0.21, 95% CI 0.11–0.42; $p < 0.001$) (Figure 1). Despite variability in study design and prevalence of CDI, there was little heterogeneity in the effect estimate ($I^2 = 0\%$; $p = 0.92$). A meta-analysis of the four studies that used *S. boulardii* showed a trend towards lower CDI rates in the probiotic group, but this result was not significant (risk ratio 0.70, 95% CI 0.29–1.69) and there was more heterogeneity ($I^2 = 17.2\%$; $p = 0.30$). A meta-analysis of all seven studies with these probiotic preparations showed a combined overall effect of lower CDI rates among antibiotic recipients randomized to probiotics than those randomized to placebo (risk ratio 0.39, 95% CI 0.19–0.79; heterogeneity of the effect estimate: $I^2 = 33.9\%$; $p = 0.17$).

3.3. Additional trials of primary CDI prevention

Three primary prophylaxis studies that did not meet our inclusion criteria, but were noteworthy nonetheless, included one randomized study that measured *C. difficile* colonization as the outcome and two non-randomized time-series analyses that measured CDI rates.

The first study randomized critically ill patients taking antibiotics in an intensive care unit setting to *Lactobacillus plantarum* 299 v or placebo and sampled stools for *C. difficile* colonization twice a week. Four of 21 placebo recipients versus none of 22 patients receiving *L. plantarum* 299 v became colonized ($p = 0.0485$).[44]

The next study encouraged physicians to write orders for a probiotic combination (*L. acidophilus* and *Bifidobacterium bifidum*) three times daily for inpatients prescribed antibiotics.[45] They documented that ~60% of eligible patients received the probiotic and also reported a lower CDI rate compared with historical rates in their hospital (10.28 cases/1000 discharges for the 2 years prior to the intervention and 3.27 cases/1000 during the year-long intervention).

The third study reported an intervention in a community hospital in Quebec that was implemented during the multi-hospital CDI outbreak in which the epidemic *C. difficile* strain BI/NAP1/027 predominated.[31] Beginning February 2004, Bio-K+ CL1285 was distributed automatically by the pharmacy department to every patient started on antibiotics and was continued for a duration of 30 days. In the first 18 months of the intervention, a 73% reduction in nosocomial CDI cases (from 18.4 to 5.0 cases/1000 admissions, $p = 0.003$) and a 94% reduction in severe CDI cases (from 5.1 to 0.3 cases/1000 admissions, $p = 0.003$) was noted. As of November 2010, over 30 000 patients have received the probiotic in this hospital and no cases of Lactobacillus septicemia have been recognized (personal communication, Dr P.J. Maziade).

4. Discussion

Results of CDI prevention studies using probiotics have not been convincing, particularly in secondary CDI prevention for patients with a recent CDI episode,[6] and the recently published Society for Healthcare Epidemiology of America (SHEA)/ Infectious Diseases Society of America (IDSA) guidelines found insufficient evidence to recommend probiotics for this indication.[46] These concerns about the lack of probiotic efficacy in secondary prevention may have influenced the criticism of probiotic trials for primary prevention. A recently reported randomized, double-blind, placebo-controlled study of a probiotic Lactobacillus/*Streptococcus thermophilus* preparation demonstrated a 22% reduction in AAD and a 17% reduction in CDI

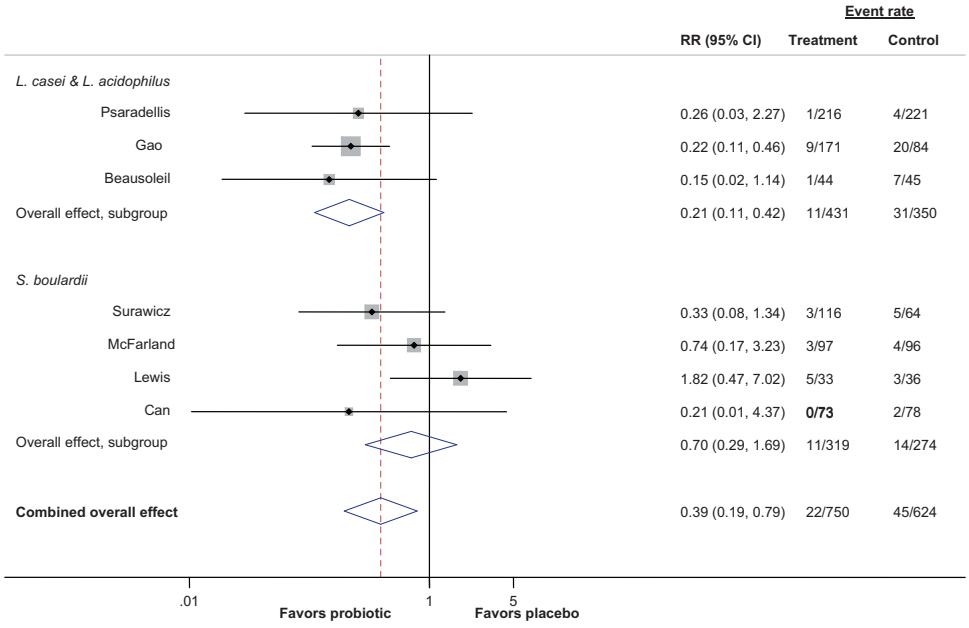

Figure 1. Meta-analysis of three randomized trials for primary prevention of CDI using *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R (Bio-K+ CL1285) and the four trials using *Saccharomyces boulardii* among antibiotic recipients. The combined overall effect for these seven trials is shown on the bottom. Relative risk (RR) and 95% confidence interval (CI) of CDI for patients taking the probiotic compared with patients taking placebo is indicated for each study. The vertical dashed line represents the point estimate of the overall RR.

among hospitalized patients taking antibiotics.[41] This study was widely criticized for questionable choice and blinding of the placebo drink and the numerous exclusions in the protocol, including high risk antibiotics, making it difficult to understand how these results, if repeatable, would be generalizable.[47,48] The results, however, were subsequently repeated in a randomized study using another Lactobacillus preparation, *L. casei* and *L. acidophilus* (Bio-K+ CL1285), that did not exclude high risk antibiotics.[10] While there are questions about the design and execution of this study as well, there are reasons to hypothesize that probiotics might be more efficacious in primary CDI prevention than in secondary prevention. We hypothesize that the efficacy of probiotics for the prevention of CDI relates to the extent of disruption of the protective host colonic microbiota.

Appreciation of the extent and diversity of the human colonic microbiota has been enhanced with the development of culture-independent techniques based on amplification of 16s rRNA.[49] Antibiotics have a profound effect on the richness, evenness, and diversity of the microbiota, even in the absence of overt gastrointestinal symptoms.[50] Chang et al. used similar techniques to study the microbiota of patients with initial CDI episodes and recurrent CDI episodes compared to controls without CDI infection.[51] The striking finding in that study was the marked redistribution of major bacterial phyla and much lower diversity of the biota among the patients with recurrent CDI. In contrast, the microbiota in patients with an initial CDI episode was more similar to that of the controls than that of the recurrent CDI patients. It is possible that the opportunity for a probiotic effect is greatest at the time of initial exposure to *C. difficile* following antibiotic disruption of the flora but before the more pervasive disruption following established infection with *C. difficile*.

Even though the majority of the randomized probiotic studies of primary prevention for CDI did not show statistically significant differences and were seriously underpowered for this outcome evaluation, the trend was towards protection in nine of the 11 studies. Our meta-analysis provided the opportunity to better understand these trends for the two best-studied probiotic formulations (*L. acidophilus* + *L. casei* and *S. boulardii*). Our findings indicate a consistent and significant effect for the *L. acidophilus* + *L. casei* formulation and a trend towards a beneficial effect for *S. boulardii* preparations; the combined overall effect showed significant protection from CDI (Figure 1).

The recent reports of primary prophylaxis attempts using the Lactobacillus preparation Bio-K+ CL1285[8–10] are particularly encouraging. The 9-month study conducted in one Montreal area hospital during the 2003–2004 BI/NAP1/027 CDI epidemic came close to showing effectiveness of this product for CDI prevention.[8] Furthermore, the analysis of the three *L. acidophilus* + *L. casei* studies and the overall combined meta-analysis of the *L. acidophilus* + *L. casei* and *S. boulardii* studies showed significant protection against CDI among antibiotic recipients who took probiotics during their time at risk. While the extraordinary rate of CDI among the placebo recipients in the two studies showing efficacy[10,41] is still incompletely explained, the possibility of primary CDI prevention using specific probiotics is intriguing and worthy of further study.

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Conflict of interest: SJ, PJM, LVM, WT, CD, BC, DEL, and ECJG have served on the advisory board of Bio-K+. This paper was conceived by the members of the advisory board and was written solely by the advisory board without input from Bio-K+.

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