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Review

Primary prevention of *Clostridium difficile* infections with a specific probiotic combining *Lactobacillus acidophilus*, *L. casei*, and *L. rhamnosus* strains: assessing the evidence

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SUMMARY

Clostridium difficile infection (CDI) has become the leading healthcare-associated infection and cause of outbreaks around the world. Although various innovative treatments have been developed, preventive strategies using multi-faceted infection control programmes have not been successful in reducing CDI rates. The major risk factor for CDI is the disruption of the normally protective gastrointestinal microbiota, typically by antibiotic use. Supplementation with specific probiotics has been effective in preventing various negative outcomes, including antibiotic-associated diarrhoea and CDI. However, a consensus of which probiotic strains might prevent CDI has not been reached and meta-analyses report high degrees of heterogeneity when studies of different probiotic products are pooled together. We searched the literature for probiotics with sufficient evidence to assess clinical efficacy for the prevention of CDI and focused on one specific probiotic formulation comprised of three lactobacilli strains (*Lactobacillus acidophilus* CL1285, *Lactobacillus casei* LBC80R, *Lactobacillus rhamnosus* CLR2, Bio-K+) for its ability to prevent CDI in healthcare settings. A literature search on this probiotic formulation was conducted using electronic databases (PubMed, Google Scholar), abstracts from infectious disease and infection control meetings, and communications from the probiotic company. Supporting evidence was found for its mechanisms of action against CDI and that it has an excellent safety and tolerability profile. Evidence from randomized controlled trials and facility-level interventions that administer Bio-K+ show reduced incidence rates of CDI. This probiotic formulation may have a role in primary prevention of healthcare-associated CDI when administered to patients who receive antibiotics.

Introduction

Clostridium difficile infections (CDI) have been a persistent and difficult clinical challenge for the past four decades. CDI has become the most widely reported healthcare-associated

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infection, accounting for >453,000 cases per year [1,2]. The ease of transmission of *C. difficile* is due to multiple factors: antibiotic-resistant spores (found on 20–50% of hospital environmental surfaces and on hands of hospital staff and patients), the ability of *C. difficile* vegetative cells to colonize patients with disrupted intestinal microbiomes (by antibiotics, surgery, or other procedures), and the production of toxins that cause cellular damage and initiate an inflammatory response leading to symptomatic CDI [3–13]. In addition, the treatments for CDI may fail to eradicate the *C. difficile* spores, leading to 20–30% of the cases to recur within two or three months. These are major reasons why CDI is so difficult to treat and why prevention of healthcare-associated CDI has been challenging. Symptoms of CDI range from mild diarrhoea to severe colitis, and complications may include sepsis, colonic perforation, or the need for colectomy [9]. Consequences of CDI include prolonged healthcare stays, 24% higher hospital readmission rates, increased risk of other nosocomial infections, higher mortality rates (as high as 22% 90-day mortality), and higher healthcare-associated costs (reaching US$4.8 billion per year) [10,14,15]. Outbreaks of CDI in hospitals and long-term care facilities are occurring with increasing frequency across the globe despite efforts to control these outbreaks [10,16]. Among 32 investigational treatments for CDI, only two are currently approved for use in CDI [17].

Efforts targeted at primary prevention of CDI have focused on multi-disciplinary infection control practices or 'bundles', which have resulted in reduced CDI rates but have been unable to eradicate CDI due, in part, to the difficulty in long-term sustainability of these enhanced infection control programmes and the varying efficacy of the different bundle components [18–20].

The search to improve infection control bundles has led to investigating the role of adding a probiotic component [21]. Because common healthcare exposures (such as antibiotics) may reduce the patient's intestinal microbial diversity resulting in osmotic and secretory changes that lead to diarrhoea, and because probiotics have been shown to help restore this disruption, this strategy seems promising [22,23]. Clinical trials have demonstrated efficacy for specific probiotics in preventing antibiotic-associated diarrhoea (AAD) and prevention of recurrences of CDI [17,23–25]. A recent Cochrane review found 'moderate quality evidence' suggesting that probiotics are both safe and effective for preventing CDI but noting a high level of heterogeneity when different studies were pooled [25]. Other meta-analyses have pooled studies of different types of probiotic products, which can result in contributing to high levels of heterogeneity and may introduce bias. Beyond specific probiotic strain(s) tested, differences in efficacy can also be a function of daily doses and formulations used [26]. In-vitro studies across *Lactobacillus* spp. strains and probiotic formulations show enormous variation in competitive mechanisms and potency. For example, significant differences in antibiotic sensitivities were found among 170 strains of lactobacilli [27]. Even when comparing 13 different probiotic products of the same strain (*Lactobacillus rhamnosus* GG), available in different formulations (four capsule products, two commercial infant foods, three from freeze-dried products, and four from soft agar), significant differences in the ability to interfere with pathogen attachment among the products were found [28].

A specific formulation of *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2 (Bio-K+) has been marketed in North America since 1996, is well characterized in terms of mechanisms of actions, safety, kinetics, and clinical efficacy in various therapeutic applications [29]. Previous meta-analyses have shown that this formulation effectively prevents CDI in patient-level randomized controlled trials [30,31]. This review examines the totality of the evidence for this specific probiotic formulation and examines its use when added to infection control bundles with new facility-level intervention studies for the primary prevention of CDI.

Methods

Search strategy

A literature search from January 1st, 2000 to January 1st, 2018 was conducted, using electronic databases (PubMed, Medline, Google Scholar, Cochrane Databases), meeting abstracts from infectious disease and infection control meetings, and communications with probiotic manufacturers. Reference lists from extracted articles or reviews were also searched. We initially searched the literature for probiotics that were being used as facility-level interventions to reduce CDI rates and restricted further searches to specific probiotics with at least two intervention trials, resulting in only one probiotic with multiple studies. The following search terms and Medical Subject Headings (MeSH) were included: ((probiotic) OR (probiotic OR Lactobacillus) OR (Bio-K+) OR (*L. acidophilus* CL1285) OR (*L. casei* LBC80R) OR (*L. rhamnosus* CLR2) OR (infection control) AND (prevention of *Clostridium difficile*)). No language restrictions were imposed.

Selection criteria

Inclusion criteria included: in-vitro studies or animal models for mechanism of action or pharmacokinetic studies, randomized controlled trials or facility-level interventions in adult or paediatric inpatients at risk for CDI, published papers or meeting abstracts of the probiotic formulation of interest. Reviews and meta-analyses were screened but not included if no new information was provided. Exclusion criteria included: probiotic not fully described (strain(s) not described, no daily dose or duration data), no control group for randomized controlled trials (no placebo group) or no pre-intervention period for facility-level studies and less than two facility-level intervention studies for prevention of CDI with the same probiotic.

Results

Literature search

Initial screening of the literature found a total of 129 articles on probiotics and prevention of CDI: 123 studies of probiotics from database searches and six from meeting abstracts. Of these, sixty-nine (reviews, commentaries, meta-analyses, duplicate studies) were excluded. Much of the evidence for reducing CDI was found from randomized controlled trials testing various probiotics for the prevention of antibiotic-associated diarrhoea, which also reported CDI rates as a secondary outcome. However, most of these trials had insufficient power to detect significant differences in CDI rates, and

probiotics with fewer than two randomized trials showing significant reduction in CDI rates were excluded ($N = 31$). Five studies of probiotics with only one facility-level intervention study per probiotic type were then excluded [32–36]. One study was included that did not report CDI rates (the paper reported cases per quarter), but CDI rates were obtained from the author and one study did not specify Bio-K+ as the probiotic used in the published paper, but communication with the authors confirmed the product and dose used [37,38]. This resulted in 24 studies of this three-strain lactobacilli probiotic: mechanism of action or pharmacokinetic studies ($N = 6$), safety studies ($N = 4$), cost-effectiveness study ($N = 1$), randomized controlled trials ($N = 3$), and facility-level intervention studies ($N = 10$).

Evidence for potential efficacy

For a probiotic to be an effective preventive bundle component for CDI, it should be able to act against the pathogen itself and to survive in adequate concentrations in the gastrointestinal tract. We found evidence that this probiotic formulation survives to the target organ and has multiple mechanisms that act to reduce the effects of CDI.

Delivery to the target organ

The probiotic microbes need to survive to the target organ in adequate numbers and resist the effects of the competitive intestinal microbiome plus the effects of common healthcare exposures, such as antibiotics. Two models (simulated gastric fluid and culture plates supplemented with up to 50 g/L of bile salts) tested survival of these strains (*L. acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2) and found that they survived well in these conditions (Table A.I, Appendix A) [39]. In another study, 29 commercially available probiotic products (capsules, fermented milks, probiotic-enriched yogurts, or powders) were also compared using these same models [40]. Bio-K+ fermented milk demonstrated the best survival rate among fermented milks or probiotic-enriched yogurts [40]. Enteric coated capsules (which maintain viability of freeze-dried microbes through acidic environments), including Bio-K+ capsules, had higher survival rates. Given the importance of survival to the intestine, the integrity of the formulation is routinely verified on the finished Bio-K+ capsules, following standard methods outlined in USP 701 [41].

Gastrointestinal survival

Detecting living probiotic microbes in the host's stool is considered a surrogate for presence within the intestines. A higher concentration of faecal lactic acid bacteria (LAB) was detected in mice given Bio-K+ (Figure A1, Appendix A) [39]. The level of lactobacilli was not different than controls nine days after the last dose. Molecular tags now exist that can distinguish the specific strains in Bio-K+ from other lactobacilli in blood and stool samples, which may allow future studies of the kinetics of these lactobacilli strains in humans [42].

Interaction with antibiotics

Antibiotics represent an important threat to the viability of living bacterial probiotics. Goldstein *et al.* described the genus *Lactobacillus* sp. as 'taxonomically complex' with important differences in susceptibilities to antibiotics by strain [27]. For example, *L. casei* and *L. rhamnosus* tend to be resistant to vancomycin, whereas *L. acidophilus* is more susceptible [27]. Ensuring that a probiotic is compatible with antibiotic therapy is an important consideration when designing controlled clinical studies among antibiotic users. In three placebo-controlled trials testing Bio-K+, patients were being treated with a wide variety of antibiotics (most usually β-lactams or macrolides), as shown in Figure A2 (Appendix A) [43–45]. One study focused on patients taking antibiotics that are associated with a higher risk of CDI, such as cephalosporin and clindamycin [45]. A precaution consistently taken in these studies was to stagger the once-daily probiotic dose 2 h apart from antibiotic administration.

Mechanisms of action

Specific probiotic strains have been found to possess multiple mechanisms of action acting at different levels, but not all probiotic strains possess all these functions. Potential targets for probiotics relating to CDI include: (i) helping to restore the normally protective intestinal microbiome; (ii) direct inhibition of *C. difficile* growth; (iii) neutralization of *C. difficile* toxins; or (iv) modulation of the inflammatory response [46,47]. In pre-clinical studies, Bio-K+ has been found to possess several mechanisms of action beneficial in the prevention of CDI.

Impact on intestinal microbiome

The intestinal microbiome is a complex ecology of microbes adapted to survive together in constant competition and synergy. In a mouse model, Bio-K+ (given over 18 days) was found to decrease levels of staphylococci and increase levels of lactobacilli (Figure A1, Appendix A), suggesting Bio-K+ can temporarily modify the faecal microbiome [39].

Direct inhibition of C. difficile growth

Bio-K+ formulations were designed to include *Lactobacillus* sp. strains with a strong capacity to compete with pathogens [29]. Each strain (*L. acidophilus* CL1285, *L. casei* LBC80R, *L. rhamnosus* CLR2) has been shown to effectively inhibit the growth of multiple pathogens *in vitro*, including meticillin-resistant *Staphylococcus aureus* (MRSA) and widespread food-borne pathogens such as *Listeria innocua*, *Enterococcus faecium*, and *Enterococcus faecalis* [48,49]. The mechanisms underlying the protective effect of Bio-K+ may be due to bacteriocin-like substances, in addition to organic acids produced by the strains in Bio-K+ [48]. When tested against pH neutralized or gamma-radiation solutions, a protective effect was still observed, indicating that both factors (acid and bacteriocin production) have a role [50]. To determine whether the strains in Bio-K+ could directly inhibit the growth of *C. difficile*, several studies were performed *in vitro* under anaerobic conditions, as described in Table A.I [29,51]. Bio-K+ effectively inhibited *C. difficile* growth; however, the potency of the supernatant exhibited little or no inhibition, suggesting that the lactobacilli strains needed to be in contact with *C. difficile* to exert their antimicrobial activity [29].

Clostridium difficile toxin neutralization

The main threat from *C. difficile* stems from the effect of its toxins on the colonic epithelium, resulting in a disruption of the cytoskeleton and loss of cellular integrity and function. The ability of cell-free supernatants (extracellular products) from the individual strains or from a Bio-K+ fermented beverage to

prevent damage induced by toxins A/B was assessed in human enterocyte-like Caco-2 and HT-29 cells [29]. The supernatants from each of the three individual Bio-K+ strains or the combination inhibited the cytotoxic effect caused by *C. difficile*, allowing cells to preserve their normal structure (Figure A.3, Appendix A). To determine whether the observed effect could be attributable to a protein, supernatants were subjected to heat (99°C) or treated with proteolytic enzymes. Neither heat treatment nor trypsin/proteinase K treatments reduced the anti-cytotoxic activity [29]. Based on Qa'Dan *et al.*'s study suggesting that an acidic environment prevents the binding of *C. difficile* toxin B to its receptor, the impact of pH of the lactobacilli supernatants was tested [52]. Above a pH of 5, no anti-cytotoxic activity was observed with the Bio-K+ supernatant. Moreover, pre-treatment of *C. difficile* supernatant with a solution containing ≥1% lactic acid prevented any cytotoxic effects of the toxins, suggesting that organic acid is at least partially responsible for toxin neutralization and the cells' protection.

To determine whether this protective effect was common to all lactic acid bacteria, supernatants from ten other strains were tested but *L. acidophilus* ATCC 832, *L. acidophilus* ATCC 53671, *L. casei* ATCC 4007, and *L. rhamnosus* ATCC 4796 did not have any anti-cytotoxic activity, demonstrating that this activity may not be present in all lactobacilli strains [51].

Evidence from randomized, placebo-controlled trials

In the early 2000s, hospitals across the province of Quebec, Canada, saw an epidemic of CDI cases and higher mortality rates attributable to CDI [16,53]. In the context of this crisis, Beausoleil *et al.* contemplated using living bacteria to restore the intestinal microbiome that had been disrupted with antibiotics [43]. To date, three randomized controlled trials have investigated Bio-K+ in patients receiving antibiotics (Table I). In one study, 89 inpatient adults receiving antibiotics at one hospital in Canada were enrolled and then randomized to either Bio-K+ or placebo given once daily for the duration of the antibiotic treatment and then followed for an additional three weeks for incident diarrhoea [43]. Bio-K+ was found to significantly reduce the incidence of AAD (7/44, 15.9%, $P = 0.03$) compared to those given placebo (16/45, 35.6%), and a trend was found for the reduction of CDI (1/44, 2.3%, $P = 0.06$) in Bio-K+ treated versus placebo (7/45, 16%). This study was underpowered for the secondary outcome (CDI) and insufficient patients developed CDI to detect a significant difference.

In a confirmatory AAD prevention study, Sampalis *et al.* enrolled more subjects ($N = 472$), but this study was also underpowered for its secondary outcome (CDI) and failed to reach significance [44]. Of 437 who completed the study, only five cases of CDI were observed; one of 216 (0.5%) in the treatment group, and four of the 221 (1.8%) in the placebo group ($P > 0.05$).

A third study (Gao *et al.*) recruited 255 elderly inpatients, aged 50–70 years, treated with high-risk antibiotics at a single centre in Shanghai, China [45]. Patients were randomized to one of three groups: a single dose of Bio-K+, a double dose of Bio-K+, or placebo, for the duration of the antibiotic and an additional five days and then monitored for an additional three weeks. In this patient population, the placebo-treated subjects had a much higher incidence of CDI (23.8%) compared to the two other clinical trials (1.8–16%). The double dose of Bio-K+ had a significantly lower incidence of CDI (1.2%, $P = 0.002$) compared to the placebo group (23.8%), as did the group

Table I
Patient-level *Clostridium difficile* infection (CDI) observed in antibiotic-associated diarrhoea prevention randomized controlled trials

Population (no. intended to treat)	Formulation	Duration	Incidence of CDI			Reference
			Controls	Probiotic	*P*-value	
Single site, inpatient adults, starting antibiotics ($N = 89$)	1 × 50 billion cfu, once daily, fermented milk	Duration of antibiotics (mean: 7–8 days) only. Follow-up: 21 days	7/45 (15.6%)	1/44 (2.3%)	0.06	[43]
Multi-site (eight hospitals), inpatient adults, starting antibiotics ($N = 437$)	1 × 50 billion cfu, once daily, fermented milk	Duration of antibiotics (mean: 12 days), plus 5 days. Follow-up: 21 days	4/221 (1.8%)	1/216 (0.5%)	NS	[44]
Single site, inpatient adults, aged 50–70 years, starting high-risk antibiotics ($N = 255$)	1 × 50 billion cfu, once daily, capsule 2 × 50 billion cfu, once daily, capsule	Duration of antibiotics (3–14 days), plus 5 days. Follow-up: 21 days	20/84 (23.8%)	8/85 (9.4%) 1/86 (1.2%)	0.03 0.002[a]	[45]

[a] CDI incidence significantly lower with 2 × 50 billion cfu vs 1 × 50 billion cfu ($P = 0.04$).

treated with a single dose of Bio-K+ (9.5%, $P = 0.03$). Based on this study, a cost-effectiveness analysis estimated that the use of one capsule per day of Bio-K+ would save US$1968 and the use of two capsules per day would result in a cost-saving of $2661/patient [54]. No serious adverse reactions to the Bio-K+ formulation were observed in any of the three trials. The pooled results from each study found a significant reduction in CDI risk compared to the controls (relative risk (RR): 0.21; 95% confidence interval (CI): 0.11–0.40), as shown in the forest plot (Figure 1) from a meta-analysis [55]. It should be noted that the follow-up in all these trials (three weeks) may have missed community-onset healthcare facility-associated (CO-HCFA) cases of CDI, which may occur for up to three months after antibiotic exposure [9,56].

Facility-level efficacy

In practice, some hospitals include a probiotic as part of a multi-component bundle of infection prevention practices for antibiotic users [30]. Whereas most aim to reduce exposure to *C. difficile* spores, a probiotic may also increase the host's resilience to infection [46].

At least seven North American hospitals have documented their experiences implementing Bio-K+ with >60,000 antibiotic users [57] (Table II). This probiotic was first implemented on a facility-level basis in 2003 during a period when hospitals in a Canadian province were experiencing large CDI outbreaks due to the ribotype NAP1/027/BI strain of CD [16,53]. After augmented standard infection control precautions had failed to abate this outbreak, one hospital added Bio-K+ as part of their preventive CDI bundle and gave every hospitalized adult receiving antibiotics this formulation, resulting in significant reductions in CDI rates (Figure 2) [58]. In subsequent studies, the objective was to reduce the risk of healthcare facility-onset CDI infection in adults taking antibiotics. Some hospitals conducted the intervention hospital-wide, whereas others restricted the use to certain wards or high-risk groups [37,38,58–63]. The doses and formulations were consistent with the doses used in the clinical trials, most often two capsules per day (1×10^{11} cfu/day). The probiotic was typically given at the onset and for the duration of the antibiotic or until discharge. Several studies continued the probiotic for five to seven days post antibiotic administration [37,60,62].

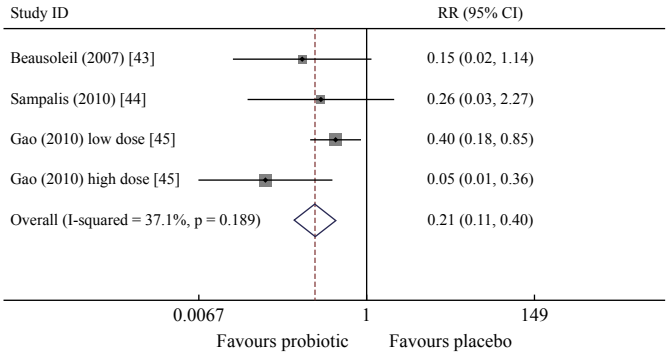

Figure 1. Forest plot of meta-analysis of three randomized, controlled trials (four treatment arms) using Bio-K+ for the prevention of *Clostridium difficile* infections. Modified from McFarland [55]. RR, relative risk; CI, confidence interval.

Of the five longitudinal studies (excluding one that did not report efficacy results [61] and one that was cross-sectional [63]), there tended to be a lower CDI rate following intervention with Bio-K+, although the change was not always statistically significant [57]. Of the two studies that only distributed the probiotic intervention on certain units or wards, both showed a significant reduction in CDI rates [38,62]. Changes in infection control practices were also occurring at both hospitals at the same time, so a causal relationship in the reduction in CDI rates cannot be directly attributed to the probiotic component. At three hospitals, the probiotic intervention was given hospital-wide rather than being restricted to certain wards [37,58–60]. At one hospital (Sharp Coronado), the CDI rates were already falling due to a change in infection control practices (antibiotic stewardship, monitoring proton pump inhibitor use, and access to probiotics on the formulary (*S. boulardii* or Lactinex)), but, as shown in Figure 2, CDI rates fell even further when Bio-K+ replaced the prior formulary probiotics [37]. At the other two hospitals (Pierre-Le Gardeur and Advocate Christ), a reduction in CDI rates was also observed [58,60]. Both maintained stable CDI preventive bundles before and during the study intervention [58,60]. At one hospital, the reduction of CDI rates was only observed during the last six months of the one-year intervention, perhaps due to 83% compliance, when administrating the probiotic [60]. The most rapid reduction of CDI rates was observed at Pierre-Le Gardeur (Lachenaie, Quebec) when the probiotic intervention was started at the peak of CDI outbreaks occurring at the time [16]. A 73% reduction in CDI rates was observed (falling from 18.4 to 3 per 10,000 person-days) after one year of probiotic administration (Figure 2) [58].

Three hospitals continued the addition of this probiotic formulation to their infection control bundles for two to 10 years [37,59,62]. Two hospitals with sustained, lower CDI rates (Figure 2) were correlated with good compliance rates (>90% eligible receiving the probiotic) at Sharp Coronado Hospital [37] and (>99% compliance) at Pierre-Le Gardeur Hospital [59] but one hospital did not report its compliance rate [62]. The rate of CDI at Pierre-Le Gardeur Hospital from 2005 to 2014 consistently remained two- to ten-fold lower than other hospitals in Quebec that did not administer Bio-K+ as part of their infection control bundle (neither another nearby local hospital, nor in 95 hospitals in a CDI surveillance programme across Quebec, nor in 27 other hospitals in Quebec of the same size (>250 bed) as Pierre-Le Gardeur Hospital [59].

Safety

The safety profile of probiotics is generally considered good compared to most medicines but is often not verified or quantified [64]. Bio-K+ fermented food products (milk, soy, rice, hemp, or pea-based) and capsules have been commercially available in Canada and the USA for >20 years. Manufacturing controls and molecular methods are employed in the production of Bio-K+ products to minimize potential risks and optimize potency [29]. Clinical research experience and epidemiological surveillance are necessary to estimate the risk of harm. Goldenberg *et al.* found in their meta-analysis of subjects taking antibiotics in randomized trials ($N = 781$) that the tolerability was good and the Bio-K+-treated subgroup had a trend for fewer adverse reactions than placebo (RR: 0.92; 95% CI: 0.76–1.1) [25]. In none of the

Table II
Facility-level distribution of probiotic to prevent nosocomial *Clostridium difficile* infection (CDI)

Population (no. probiotic-treated)	Formulation	Duration	CDI rate			Reference
			Initial	Later date	*P*-value	
Hospital-wide, inpatient adults, starting antibiotics ($N = 44,835$) (Pierre-Le Gardeur Hospital)	2 × 50 billion cfu, once daily, capsule[a]	Duration of hospital stay	91/1580 (5.8%) 18.4 per 1000 admissions	77/4968 (1.5%) 2 or 3 per 10,000 patient-days	<0.05 <0.05	[58, 59]
General medicine and surgery, inpatient adults, aged >50 years, starting broad spectrum antibiotics ($N = 6333$) (St Joseph Health Centre)	2 × 50 billion cfu, once daily, capsule	Duration of antibiotics plus 5 days	1.1 per 1000 patient-days	0.6 per 1000 patient-days	<0.05	[62]
Hospital-wide and long-term care facility, adults, starting antibiotics (*N*: not reported) (Sharp Coronado Hospital)	2 × 50 billion cfu, once daily, capsule	Duration of antibiotics plus 7 days	15 cases per quarter	2 or 3 cases per quarter	NR	[37]
Hospital-wide, inpatient adults (except oncology), starting antibiotics ($N = 360,016$ patient-days) (Advocate Christ Hospital)	2 × 50 billion cfu, once daily, capsule	Duration of antibiotics, plus 5 days	7.7 per 10,000 patient-days	8.0 per 10,000 patient-days	NS[b]	[60]
Vascular—thoracic ICU, inpatient adults, starting antibiotics and high CDI risk ($N = 61$) (Florida Hospital)	2 × 50 billion cfu, once daily, capsule	Duration of antibiotics	14.7 per 10,000 patient-days	3.1 per 10,000 patient-days	0.025	[38]
Two internal medicine units, inpatient adults ($N = 116$) (Enfant Jesus Hospital)	2 × 50 billion cfu, once daily, capsule[c]	Duration of antibiotics	41.2 per 10,000 patient-days		NA[d]	[61]
Hospital-wide, inpatient adults, starting IV antibiotics ($N = 649$) (Scripps Memorial Hospital)	1 × 50 billion cfu, twice daily, capsule	Duration of antibiotics	19 cases per 1576 patients (1.2%)		NA[e]	[63]

cfu, colony-forming units; IV, intravenous route; NA, not applicable; NS, non-significant, NR, not reported.
[a] Previous dosing was 1 × 50 billion cfu fermented milk, followed by 2 × 30 billion cfu capsules. Presently adults (aged <50 years) receive 1 × 50 billion cfu capsule.
[b] During second half of the probiotic year (months 7–12), CDI rate significantly fell compared to baseline (5.9 per 10,000 patient-days, $P < 0.05$).
[c] In feeding-tube patients, dose was 1 × 50 billion cfu, once daily, fermented milk.
[d] Statistical comparison not done.
[e] Retrospective, cross-sectional study.

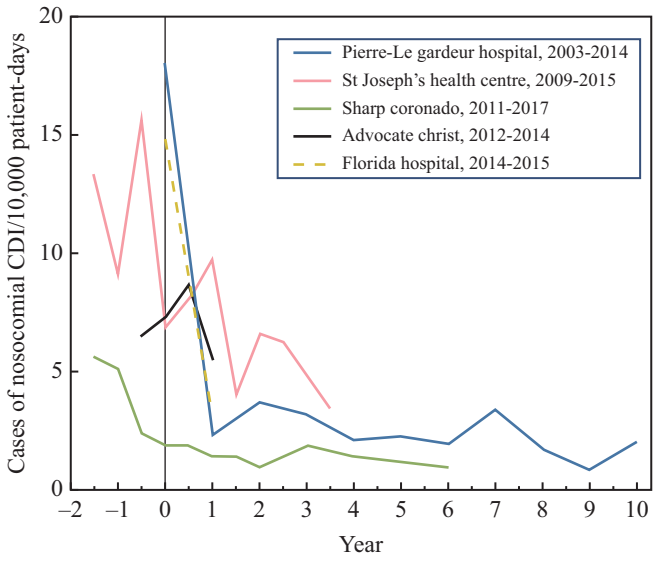

Figure 2. Longitudinal changes in the rate of nosocomial *Clostridium difficile* infection (CDI) in hospitals that have implemented Bio-K+ programmes. Each data point represents the average rate of the preceding six months (for example, the data point at baseline, year 0, represents the average rate of nosocomial CDI in the preceding six months) [57]. For Pierre-Le Gardeur Hospital, the rates of CDI in the first year were converted from cases per 1000 patient admissions using historical data [59]. For Sharp Coronado Hospital [37], CDI incidence for years 4, 5, and 6 and correct dose data were obtained by personal communication from B. Olson. Bio-K+ dosing at the Florida Hospital was confirmed as two capsules of 50 billion colony-forming units of Bio-K+ once daily, by personal communication with P. Louzon [38].

three randomized clinical trials or facility-level programmes have more adverse reactions been noted in those treated with Bio-K+ than in control groups.

Salminen *et al*. gathered data from a centralized national testing laboratory in Finland and found that *Lactobacillus* sp. bacteraemia was rare (0.2%) [65]. Using molecular methods, this laboratory also confirmed that, over the span of six years and 25 million litres of *L. rhamnosus* GG fermented milk ingested, only 11 of the *Lactobacillus* sp. bacteraemia cases occurred with the probiotic strain. Boumis *et al*. reviewed the literature and found 10 cases of *L. rhamnosus* endocarditis in patients consuming probiotics, but they could not document that the strain consumed was identical to the *L. rhamnosus* strain causing the endocarditis [66]. No cases of bacteraemia from a Bio-K+ probiotic strain have been observed or reported, neither in published observational studies nor reported through product surveillance. Aroutcheva *et al*. published a case report of a 69-year-old man who received Bio-K+ and developed *Lactobacillus* sp. bacteraemia, but molecular methods confirmed that the sepsis was not due to any of the three lactobacilli strains found in Bio-K+ [42].

Discussion

There is a dire need for strategies to prevent CDI, which may include the use of specific probiotics administered with prescribed antibiotics to inpatients. The probiotic described in this review, Bio-K+ (comprised of *L. acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2), has a solid foundation of clinical evidence supporting its efficacy in the primary prevention of CDI and valuable pre-clinical data to explain how this occurs. These bacteria employ multiple mechanisms directed against *C. difficile* and are formulated to survive to the target organ. An evidence base of three randomized clinical trials and seven observational studies document the use of Bio-K+ for CDI prevention with targeted patients (patient-level) or to all at-risk patients (facility-level) with no serious adverse effects.

In 2012, the Natural Health Products Directorate Canadian regulatory body licensed two uses for Bio-K+ (50 billion cfu capsules): to reduce the risk of antibiotic-associated diarrhoea and CDI in hospitalized adults [67]. This is the first regulatory issuance for the primary prevention of CDI for any class of medication in Canada (Bio-K+ 50 billion cfu, NPN #80038453).

The benefits of using an effective probiotic formulation at a facility-level may extend beyond reduced CDI rates and lead to reductions in other types of diarrhoeal diseases. AAD, a frequently occurring consequence of antibiotics and a disrupted intestinal microbiome, could be preventable with specific probiotics, including Bio-K+ [22,25,47]. Fringe benefits of reducing non-CDI cases of diarrhoea may include less transmission of *C. difficile* spores from asymptomatic carriers, reduced *C. difficile* toxin testing, less prophylactic patient isolation, and shorter lengths of hospital stay [53,68]. The primary outcome in the three randomized trials with Bio-K+ was to reduce the incidence of diarrhoea in hospitalized antibiotic users [43–45]. Each trial found a reduction in AAD incidence, and a meta-analysis found a pooled reduction in risk (RR: 0.59; 95% CI: 0.42, 0.81) [25].

Efforts to prevent CDI at healthcare facilities have included enhanced multi-disciplinary infection control practice bundles, which have reduced CDI rates by 45% to 85%, but a consensus on the best combination of components has yet to be reached [69]. To further reduce CDI rates, several types of probiotic (*Saccharomyces boulardii* CNCM I-745, *L. plantarum* 299v, *L. casei* Shirota, and a mix *of L. acidophilus*, *Bifidobacterium longum* and *B. bifidum* Bb12 and another mix of eight strains, VSL#3) have been tested in either specific wards/units or in hospital-wide studies, with varying success [32–36]. However, confirmatory studies on a facility-wide programme of these types of probiotic need to be conducted. By contrast, multiple facility-wide studies indicate that adding Bio-K+ to standard infection control bundles may lead to reductions in CDI rates that may be sustained over years, even when taking into account differences in types of infection control bundle used, differences in compliance rates, and scope of administration.

Limitations found when reviewing the studies include the lack of randomized, placebo-controlled trials with the prevention of CDI as the primary outcome. All of the efficacy evidence from patient-level data are from randomized trials to prevent AAD with CDI as a secondary outcome and were mostly under-powered for this outcome. The studies of facility-level efficacy for this probiotic formulation were not from randomized controlled trials, rather from quasi-experimental design that may, however, mimic 'real-life' situations more than rigorously conducted randomized trials.

Conclusion

The probiotic formulation containing these three lactobacilli strains (*L. acidophilus* CL1285, *L. casei* LBC80R,

L. rhamnosus CLR2, Bio-K+) is a promising example of how a probiotic was used to prevent serious healthcare-associated infections such as CDI. This probiotic is well characterized, has an excellent safety profile, and, when applied in hospitals, showed reductions in CDI rates, sometimes sustained over years. More research is recommended for probiotics in the prevention of CDI.

Conflict of interest statement

Bio-K Plus International, Inc., owns and manufactures the product Bio-K+. L.V.M. is a paid lecturer for Lallemand and Biocodex and on the Biocodex Microbiome Foundation Board and a member of the Scientific Advisory Board for Bio-K Plus. N.S., J.A. and M.M. are employees of Bio-K Plus International.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jhin.2018.04.017

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