Prevention of *Clostridium difficile* Infection With Probiotics

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Despite advances in the diagnosis and treatment of *Clostridium difficile* infection (CDI) and prevention efforts to reduce the spread of *C. difficile*, CDI remains a significant challenge to healthcare systems worldwide. Further advances in prevention of CDI may need to focus on those who continue to be exposed to the organism and who are susceptible. Interventions directed toward this susceptible population, particularly hospitalized patients who receive antibiotics, may be effective. There is moderate evidence on the effectiveness of probiotics to prevent primary CDI, but there are few data to support use in secondary prevention of recurrent CDI. This review discusses the literature available on the use of probiotics to prevent primary and secondary CDI.

**Keywords.** *Clostridium difficile*; probiotics; meta-analysis.

Despite advances in the diagnosis and treatment of *Clostridium difficile* infection (CDI), prevention of CDI, particularly in the healthcare setting, has remained a challenge. A recent point prevalence survey of healthcare-associated infections (HAIs) in randomly selected inpatients from 183 hospitals in 10 states was performed in 2011 [1]. Among the 11,282 patients included, 4.0% had 1 or more HAIs. *Clostridium difficile* was the most commonly identified pathogen, accounting for 12% of all HAIs. The conclusions were that public health surveillance and prevention activities should continue to address CDI [1]. Most infection control strategies have placed emphasis on interruption of horizontal transmission of *C. difficile* between patients, their environment, and healthcare workers. Further improvements in prevention of CDI will likely need to focus on prevention of disease in those patients who continue to be exposed to the organism. Antibiotic stewardship is one potentially effective strategy but another, less studied approach is to minimize the complications of antibiotic administration using effective probiotics.

A large degree of skepticism remains about the efficacy of probiotics in prevention of CDI. Much of this skepticism likely stems from the experience of probiotics in the setting of established CDIs. It has become increasingly clear that the indigenous microflora of the intestine plays a major protective role for many enteric infections, but most notably for *C. difficile*. Patients with established CDI, particularly patients with recurrent CDI, have markedly disrupted intestinal flora. Using nonculture techniques to elaborate the fecal microbiome, Chang et al showed a progressive loss of diversity and dropout of major bacterial groups in patients with recurrent CDI [2]. In this study, patients with initial CDI episodes had distributions of major bacterial groups that more closely resembled the normal controls than patients with recurrent CDI. The rarefaction curves (measure of bacterial diversity) of the patients with initial CDI episodes overlapped those of the controls, whereas the curves of the recurrent CDI patients were markedly different [2]. We hypothesized 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 CDI in whom the indigenous intestinal microbiota is markedly disrupted [3].
Another source of confusion in the literature has been that most previous reviews and meta-analyses of probiotic studies have combined primary and secondary CDI prevention data, which potentially obscures differences between these populations. In this review, we first examine meta-analyses and comparative studies that specifically study probiotics for primary prevention of CDI in patients at risk for CDI by virtue of antibiotic use, but who have not had recent prior CDI episodes. Second, we also review the available data for secondary CDI prevention with probiotics in patients who have already had CDI and who are at risk for recurrent CDI.

**PRIMARY CDI PREVENTION**

Several systematic reviews and meta-analyses have been conducted in the past decade on the primary prevention of CDI using probiotics [3–7]. The 3 most recent studies included meta-analyses and varied in their study selection, inclusion criteria, and probiotic strains included [3, 6, 7]. However, they all concluded that the use of probiotics in patients taking antibiotics was associated with a reduction in CDI.

Johnson et al conducted a systematic review and meta-analysis of published randomized controlled trials (RCTs) that addressed primary prevention of CDI in adults as at least 1 of the outcomes [3]. Eleven studies were identified in which adult patients were randomized to a probiotic or a probiotic combination vs placebo. The criterion for the meta-analysis was that the probiotic preparation had to have been studied in at least 2 RCTs. At the time of review, 2 probiotic preparations had been evaluated in >1 RCT: the *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus* probiotic combination (Bio-K Plus International, Laval, Quebec) (3 RCTs) and *Saccharomyces boulardii* (4 RCTs) [8–14]. An overall protective effect against CDI was seen in this meta-analysis (relative risk [RR], 0.39; 95% confidence interval [CI], 0.19–0.79; Figure 1) [3]. Subgroup analyses of 3 RCTs for the *L. acidophilus* + *L. casei* + *L. rhamnosus* formulation compared with placebo also showed a protective effect (RR, 0.21; 95% CI, 0.11–0.42), whereas 4 RCTs assessing the effect of *S. boulardii* were not statistically significant but still showed lower rates of CDI (RR, 0.70; 95% CI, 0.29–1.69) [3].

This meta-analysis included an RCT by Gao et al that was conducted in a single hospital in Shanghai over a 3-month period in 2009 using the *L. acidophilus* + *L. casei* + *L. rhamnosus* formulation [9]. This study showed extremely high CDI rates in the placebo group (23.8%), similar to the high and unexpected rate (17%) seen in the study by Hickson et al [15]. Few data are
available on the epidemiology of CDI in China, and rates reported in the available studies do not suggest higher rates as seen in North America or Europe [16,17]. In addition, baseline data were unavailable in the Gao et al study, and no other data have been made available to determine trends in overall incidence of primary CDI in this facility. With exclusion of the Gao study, the subgroup analysis of L. acidophilus + L. casei + L. rhamnosus formulation still showed lower CDI rates among those taking this formulation compared with the placebo group (RR, 0.19; 95% CI, .04–.85) (Dr William Trick, personal communication).

The systematic review and meta-analysis by Johnston et al focused on the effect of probiotics on CDI and adverse events and demonstrated a reduction in CDI in those receiving probiotics (pooled RR, 0.34; 95% CI, .24–.49; Figure 2) [6]. This meta-analysis differed from the meta-analysis of Johnson et al in that it included studies (1) with adult or pediatric patients; (2) reported in abstract form; and (3) with a variety of probiotic formulations (ie, L. acidophilus + L. casei + L. rhamnosus formulation, L. rhamnosus GG, S. boulardii, and other mixed species), and it excluded the study by Lewis et al [13] because, according to the authors, it did not specify how many participants in each group developed CDI. Notable findings in this meta-analysis came from several subgroup analyses. CDI reductions were seen among those receiving multiple- or single-species probiotic formulations; however, there was a possible increased effect with probiotics in formulations using multiple species vs single species (interaction P = .06). Subgroup analyses also found significant risk reductions in CDI among those who received S. boulardii (RR, 0.39; 95% CI, .19–.82) and the L. acidophilus + L. casei + L. rhamnosus formulation (RR, 0.21; 95% CI, .11–.42), but not in those who received L. rhamnosus GG (RR, 0.63; 95% CI, .30–1.33). In all, 20 studies were included in the final meta-analysis for CDI as an outcome [6,8–12,14,15,18–29]. A later Cochrane review (n = 23 studies) assessing the effect of probiotics on several outcomes, including CDI, found lower rates of CDI in patients taking probiotics vs those in control groups (RR, 0.36; 95% CI, .26–.51; Figure 3) [7–12,14,15,18–32]. Subgroup analyses also showed significant heterogeneity by species (P = .03).
A study worth mentioning that was not included in any of the published systematic reviews or meta-analyses cited above was an RCT on whether a probiotic formulation of *L. rhamnosus* GG compared with placebo could reduce the incidence of ventilator-associated pneumonia in mechanically ventilated patients [33]. A secondary outcome included CDI, and the authors found that patients treated with probiotics had a lower incidence of CDI than those receiving placebo (5.8% vs 18.6%; *P* = .02) [33]. Not all of the patients in this study, however, received antibiotics, and as such this study is not directly comparable to the other studies included in these meta-analyses.

Since the publishing of these meta-analyses, a recent RCT from the United Kingdom assessed the effect of a probiotic formulation containing 2 strains of *L. acidophilus* (CUL60 and CUL21) and 2 strains of *Bifidobacterium* (*Bifidobacterium bifidum* CUL20 and *Bifidobacterium lactis* CUL34) compared with placebo [34]. These investigators found no significant difference between the probiotic group and the placebo group for antibiotic-associated diarrhea (RR, 1.04; 95% CI, .84–1.28) or CDI (RR, 0.71; 95% CI, 0.35–1.47). A key feature of this study however, was the low incidence of CDI overall, which occurred in <1% of the study sample [34]. This low incidence setting of CDI may be due to key antimicrobial stewardship interventions established in the UK National Health Service over the past decade [35]. Another RCT from the United Kingdom focused on a specialty population—persons with spinal cord injury—and assessed the use of probiotics in the prevention of antibiotic-associated diarrhea in patients with spinal cord injury receiving antibiotics [36]. They found that patients who received a *L. casei* strain *Shirota* probiotic drink had a lower incidence of antibiotic-associated diarrhea than controls (17.1% vs 54.9%, respectively; *P* < .001). However, because of the low incidence of CDI (only 1 case in the control group), the investigators were unable to evaluate the effects on prevention of CDI [36].

**LIMITATIONS OF PRIMARY PREVENTION STUDIES**

There are key limitations to the meta-analyses and the studies included beyond biases within the trials themselves, such as how the randomization was conducted, blinding, and missing data. Most studies were not powered sufficiently to detect a significant difference at the .05 level; only 2 studies in the Johnson et al meta-analysis had >60% power to detect a significant difference at *P* < .05 [3]. In the Cochrane meta-analysis with 23 studies included, there was still a rare number of CDI events.
(n = 154), and the calculated optimal information size of 3024 was more than the total sample size (n = 961) included in the meta-analysis [7]. In addition, the low-incidence settings of some studies made it nearly impossible to assess the association between probiotic use and CDI [34,36]. There was also variation in probiotic formulations that may suggest different effects with specific formulations, dosage, or multiple species [3,6]. The analysis of trials that included both adults and children suggest that there is heterogeneity, and should be explored further [7]. Finally, there was variability in the types of antibiotics patients were treated with in these trials. However, none of these meta-analyses evaluated the impact that antibiotic type or class could have on the efficacy of probiotics. It is unclear whether probiotics may be more or less beneficial for patients receiving certain antibiotic classes, particularly those that have been associated with the highest risks for CDI such as clindamycin, cephalosporins, and fluoroquinolones.

SECONDARY CDI PREVENTION

As mentioned in the introduction, there are theoretical reasons that secondary prevention of CDI is a more challenging hurdle for probiotics than is primary prevention. *Saccharomyces boulardii* has been the most extensively studied probiotic for secondary CDI prevention, and 2 double-blind, placebo-controlled trials were conducted to assess efficacy in this setting [37,38]. The first study included patients with an initial CDI episode as well as patients with 1 or more prior episodes. The overall results showed a lower RR of subsequent CDI in patients randomized to standard antibiotics and *S. boulardii* for 4 weeks than in those who received standard antibiotics alone (RR, 0.43; 95% CI, .20-.97) [37]. Heterogeneity was identified if the patient had a history of CDI. There was no significant difference in outcome for those with initial CDI (CDI treatment plus *S. boulardii* vs treatment plus placebo: 19.3% vs 24.2%; *P* = .86); however, in those with a history of CDI, the rate of subsequent recurrence was lower in the *S. boulardii* group compared with placebo (34.6% vs 64.7%, respectively; *P* = .04) [37]. The study was underpowered to show a difference in the patients with an initial CDI episode. The role of probiotics in secondary prevention of patients with initial or early recurrences could be readdressed in further studies of sufficient size.

A subsequent study using *S. boulardii* was designed to include only patients with recurrent CDI episodes and to control for the dosing and type of antibiotic used [38,39]. Overall, the results were disappointing as no benefit was shown for patients randomized to *S. boulardii*. A substudy of the overall trial, however, did suggest a potential benefit for those patients who were randomized to high-dose vancomycin and *S. boulardii* [38]. Three of 18 patients (17%) randomized to high-dose vancomycin (2 g/day) for 10 days and *S. boulardii* (1 g/day) for 28 days had subsequent recurrences, compared with 7 of 14 patients (50%) randomized to vancomycin alone (*P* = .05) [38]. There was no benefit to the patients randomized to low-dose vancomycin (500 mg/day) and *S. boulardii* or to those randomized to metronidazole and *S. boulardii*. A subsequent analysis of patients in these clinical trials found that vancomycin cleared *C. difficile* (by stool culture or toxin testing) at the end of the antibiotic treatment better than metronidazole, but these microbiologic data could not explain the difference in recurrence rates between high- and low-dose vancomycin [40]. In summary, the promising results of the first randomized trial of *S. boulardii* for secondary CDI prevention [37] were not duplicated in the second randomized trial of patients with recurrent CDI [38], the subgroup in the first trial that showed the most potential benefit.

Another randomized trial of interest, although stopped early for inadequate recruitment of patients, compared metronidazole (1200 mg/day) for 10 days and *Lactobacillus plantarum 299v* (5 × 10¹⁰ colony-forming units/day) for 38 days to metronidazole and placebo for patients with recurrent CDI [41]. Subsequent recurrences were seen in 4 of 11 patients (36%) randomized to metronidazole and *L. plantarum 299v* and in 6 of 9 patients (67%) randomized to metronidazole (*P* = not significant; power = 13.9%). It is possible that other probiotics or biotherapeutic agents will be more effective in secondary prevention. A nontoxigenic strain of *C. difficile* (VP20621) has been developed and is undergoing clinical trials [42]. Preliminary results from a phase 2 trial show promise for VP20621, which was given as adjunctive treatment following standard antibiotic treatment for CDI. The recurrence rate was only 2% among patients who were successfully colonized with VP20261 [43].

CONCLUSIONS

Overall, the limitations and findings from the meta-analyses and RCTs suggest that there is moderate evidence on the effectiveness of probiotics to prevent primary CDI, but there are insufficient data to support use in secondary prevention of recurrent CDI. Additional studies of sufficient size are needed to further evaluate secondary prevention. There is also a need to determine whether antibiotic class or type modifies the effect of probiotic use.

The curbing of antibiotic use is important for containing the development of CDI; however, treatment for infections is inevitable in certain patients. The use of probiotics as an adjunctive therapy may provide a key intervention in reducing primary CDI.

Notes

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