American Gastroenterological Association Institute Guidelines on the Role of Probiotics in the Management of Gastrointestinal Diseases

Grace L. Su[1,2], Cynthia W. Ko[3], Premysl Bercik[4]; Yngve Falck-Ytter[5,6], Shahnaz Sultan[7], Adam Weizman[8], Rebecca Morgan[9]

[1] Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, Michigan, U.S.A. Place, City, Country; [2] Gastroenterology Section, Veterans Administration Ann Arbor Healthcare System, Ann Arbor, Michigan, USA; [3]Division of Gastroenterology, University of Washington School of Medicine, Seattle, WA; [4]Division of Gastroenterology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada; [5]Division of Gastroenterology, Case Western Reserve University, Cleveland, Ohio, USA; [6]Louis Stokes VA Medical Center, Cleveland, Ohio, USA; [7]Division of Gastroenterology, University of Minnesota, Minneapolis, MN, USA; [8]Division of Gastroenterology, Mount Sinai Hospital, Department of Medicine, University of Toronto; [9] Department Health Research Methods, Evidence and Impact, McMaster University, Hamilton, ON

Acknowledgements: Clinical Guidelines Committee included Karen Chachu MD, PhD, Lukejohn Day MD, AGAF, Benjamin Lebwohl MD, AGAF, Thiruvengadam Muniraj MD, PhD, MRCP, Amit Patel MD, Anne F. Peery, MD Raj Shah, MD, Shahnaz Sultan, MD, Chair, Harminder Singh, MD, Siddharth Singh, MD, Stuart Spechler, MD, Grace Su, MD, Aaron P. Thrift, PhD Jennifer M. Weiss, MD and Adam V. Weizman, MD.

This document presents the official recommendations of the American Gastroenterological Association (AGA) on the XX of XX. The guideline was developed by the AGA's Clinical Practice Guideline Committee and approved by the AGA Governing Board.

1. ***Disclose conflicts of interest:***

Conflict of interest disclosure: All members were required to complete disclosure statement. These statements are maintained at the American Gastroenterological Association Institute (AGA) headquarters in Bethesda, Maryland and pertinent disclosure are published with the report.

Within the last twenty years, there has been an increasing recognition and interest in the role of the gut microbiome in gastrointestinal health[1]. Defined by the Food and Agriculture Organization of the United Nations and the World Health Organization as "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[2], probiotics hold the promise of an effective way to alter the microbiome for our benefit. Enthusiasm and popularity within the community for probiotics has led to a multi-billion-dollar industry worldwide[3]. Because probiotics are not considered drugs in the United States or Europe, the regulatory status is not the same as one which would normally accompany a pharmaceutical product. The industry is largely unregulated and marketing of product is often geared directly at consumers without providing direct and consistent proof of effectiveness[4, 5]. This has led to widespread use of probiotics with confusing evidence for clinical efficacy. It is estimated that 3.9 million American adults used some form of probiotics or prebiotics (nutrients which promote growth or beneficial functions of beneficial microbes)[6] in 2015, an amount which is four times that in 2007[7, 8]. Given widespread use and often biased sources of information, it is essential that clinicians have objective guidance for their patients about the appropriate use of and indications for probiotics.

Although there has been a substantial number of studies examining probiotics in various gastrointestinal diseases, the studies have been extremely varied including differences in the strain of microbe(s) used, dose, and route of administration as well as the research methodology, including differences in the reporting of endpoints and outcomes[4]. Furthermore, most of the studies with probiotics involved relatively small number of patients compared to trials investigating the effects of pharmacological interventions.

Conclusions drawn from meta-analyses or systematic reviews can be misleading if different studies with different patient populations, different reported endpoints and outcomes or different strains or combinations of probiotics are grouped together inappropriately[2]. Within species, different strains can have widely different activities and biologic effects. Many immunologic, neurologic and biochemical effects of gut microbiota are likely not only to be strain specific but also dose specific[5]. Furthermore, combinations of different microbial strains may also have widely different activity as some microbial activities are dependent on interactions between different strains. In developing this guideline, we have examined the evidence presented in the accompanying technical review with these constraints in mind.

This guideline was developed utilizing a process previously outlined[9]. Briefly, the AGA process for developing clinical practice guidelines follows the GRADE approach[9] and best practices as outlined by the National Academy of Science (formerly Institute of Medicine)[10]. *A priori*, the guideline development panel and methodologist identified and formulated clinically relevant questions about the use of probiotic formulations for the prevention and treatment of gastrointestinal diseases (not prebiotic use). Each research question identified the population, intervention, comparison, and patient-important outcomes. A technical review panel initially reviewed and assessed relevant systematic reviews that addressed the clinical questions, updating high quality systematic reviews through December 2018 to inform the recommendations when possible (Technical review, unpublished). For situations in which there was either no recent systematic review available or the recent systematic review was not deemed high quality, the technical review panel conducted the systematic review *de novo*. The findings from each systematic review were assessed using the GRADE approach and presented in an evidence profile. The guideline panel and the authors of the technical review met face-to-face on May 9, 2019 to discuss the findings from the technical review and formulated these recommendations. Although the quality of evidence (Table 1)

was a key factor in determining the strength of the recommendations (Table 2), the panel also considered the balance between the benefits and harms of the interventions, as well as patients' values and preferences, resource use (i.e. cost), health equity, acceptability, and feasibility. The recommendations, certainty of evidence, and strength of recommendations are summarized in Table 3. We hope to provide clinicians with clear guidance regarding the appropriate use of specific probiotics in the context of specific gastrointestinal diseases.

In addition, we were not able to assess the viability of each formulation reported in the studies as this information was not routinely available. We recognize that different manufacturers use different processes which may affect the actual content of the probiotic utilized but this is not within the scope of this guideline and thus we provided the granular data regarding each strain as specified in the published reports.

In patients with *Clostridium difficile* infection, we recommend the use of probiotics only in the context of a clinical trial.

GRADE: *No recommendation, knowledge gap*

The AGA makes no recommendations for the use of probiotics in the treatment of *Clostridium. difficile (C. difficile*) infection. Incidence of *C. difficile* infection is rising, being responsible for almost half a million infections in the United States in 2011[11], with recurrences ofup to 19.9%, and leading to 29 000 deaths. Fecal microbiota transplantation is highly effective in treating recurrent *C. difficile* infection[12], but the data supporting the use of probiotics in initial or recurrent *C. difficile* infection are less convincing.

The technical review identified five placebo-controlled RCTs evaluating probiotics as adjunct treatment with antibiotics, testing four different probiotic formulations. The patient populations across studies differed, including patients with an initial *C. difficile* infection, recurrent infection or both. Probiotics vs placebo were administered together with metronidazole or vancomycin at low-dose or high-doses. Due to these variations in the study design, as well as in clinical outcomes, data were deemed too heterogeneous to be pooled in the analysis. All five published studies contained uncertain or high risk of bias regarding blinding of outcome assessment and selective reporting.

The probiotic formulations studied included *Saccharomyces boulardii (S. boulardii), Lactobacillus plantarum (L. plantarum)* 299v, *Lactobacillus rhamnosus* GG and the 4-strain combination of *Lactobacillus acidophilus (L. acidophilus)* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium animalis (B animalis)* subsp. *lactis* Bi-07 and *B. animalis* subsp. *lactis* Bl-04. The largest study involving 134 patients found that *S. boulardii* may have a beneficial effect on cessation (RR, 1.33; 95% CI, 1.02-1.74) and recurrence of diarrhea (RR, 0.59; 95% CI, 0.35-0.98) but the quality of evidence was low. The smaller trials with *L. plantarum* 299v or the 4-strain combination suggested that these probiotics also may have beneficial effects on diarrhea but the evidence was very uncertain, while the administration of *L. rhamnosus* GG resulted in increased recurrence of *C. difficile* infection compared to placebo (RR 2.63, 95% CI: 0.35-19.85). The overall certainty of evidence across all critical outcomes for probiotics used as adjunctive treatment for *C. difficile* infection was low. Furthermore, the technical review identified a potential risk of publication bias due to multiple registered trials that were not linked to a published report. While currently available data suggest that some probiotics might be beneficial in treatment of *C. difficile*, further studies with standardized study design and larger number of patients are needed to define those probiotics, as well as to identify which patient populations may benefit from this intervention.

In adults and children on antibiotic treatment, we suggest the use of *S. boulardii*; or the two-strain combination of *Lactobacillus. acidophilus* and *Lactobacillis. casei*; or the three-strain combination of *Lactobacillus. acidophilus, Lactobacillus. delbruekii* subsp. *bulgaricus*, and *Bifidobacterium. bifidum*; or the four-strain combination of *L. acidophilus, L. delbruekii* subsp. *bulgaricus, B. bifidum*, and *Streptococcus salivarius* subsp. *thermophilus* over no or other probiotics for prevention of *C. difficile* infection.

GRADE: *Conditional recommendation, low quality of evidence*

Comment: Patients who place a high value on the potential harms (particularly those with severe illnesses) or a high value on avoiding the associated cost and a low value on the small risk of C. *difficile* development (particularly in the outpatient setting), would reasonably select no probiotics.

The AGA suggests the use of certain strains and strain combination of probiotics in the prevention of C. *difficile* infection. Although there is a large body of literature studying the role of probiotics in preventing antibiotic-associated *C. difficile* infection, the studies are very heterogeneous. The technical review identified 39 studies that were previously evaluated by Cochrane review published in 2017[13]. A total of 9,955 patients were included but the populations studied were extremely varied including pediatric, adult and elderly patients utilizing a variety of antibiotic regimens in both inpatient and outpatient settings who have very different risks for the development of *C. difficile* infection. The Cochrane review found that probiotics reduced the overall risk of *C. difficile* infection versus placebo (RR, 0.40; 95% CI: 0.30-0.52); however, the beneficial effect was driven by the population of patients with high risk of developing *C. difficile* infection, with no significant effects observed in patients with low or baseline risk.

The technical review did not identify any new RCT since the 2017 Cochrane review to November 2018 and thus assessed the certainty of evidence from these 39 trials[ref technical review]. The overall certainly

of the evidence was downgraded from Moderate to Low due to unclear or high risk of bias in most of the trials across all domains for all outcomes assessed. Several studies were published as abstracts only or referenced unpublished data. Publication bias was considered as a large number of registered trial protocols on this topic were not associated with subsequent peer-reviewed publications.

Subgroup analyses of individual probiotic strains or strain combinations which may have effect vs placebo found that the risk of *C. difficile* infection development was reduced by *S. boulardii* (RR, 0.41; 95% CI: 0.22-0.79), the two-species combination of *L. acidophilus* and *Lactobacillus casei (L. casei)* (RR, 0.22; 95% CI: 0.11-0.42), the three-strain combination of *L. acidophilus, Lactobacillus delbruekii (L. delbruekii)* subsp. *bulgaricus*, and *Bifidobacterium bifidum B bifidum*)(RR, 0.35; 95% CI: 0.15-0.85), as well as the four-strain combination of *L. acidophilus, L. delbruekii* subsp. *bulgaricus, B. bifidum,* and *S. salivarius* subsp. *thermophilus* (RR, 0.28; 95% CI: 0.11-0.67), with the overall quality of the evidence rated as Low. It should be pointed out that beneficial effect of probiotics was mainly seen in patients with very high risk of developing *C. difficile* infection (> 15% baseline risk) and that the analysis of most studies had a wide confidence interval that includes the potential for some benefit, as well as for some harm. Thus, patients who place high value on avoiding associated financial cost or potential harms (especially those immunocompromised patients) and who have low risk of developing *C. difficile* infection (mainly outpatients in the community) may choose not to use any probiotics.

In patients with Crohn's disease, the AGA recommends use of probiotics only in the context of a clinical trial.

GRADE: *No recommendation, knowledge gap.*

The AGA recommends use of probiotics on only in the context of a clinical trial for adults and children with Crohn's disease. Alterations in the gut microbiome in patients with Crohn's disease are increasingly being explored, and interest in microbiota-based therapies such as probiotics and fecal microbiota transplantation is growing. However, studies of probiotics for induction or maintenance of remission in Crohn's disease have been limited by small sample sizes, heterogeneity in patient populations, heterogeneity in study design, and differences in the probiotic formulations tested.

The technical review searched for studies of both induction and maintenance of remission in Crohn's disease in adults and children. Only one study of 11 subjects was identified for induction of remission in either adults or children. This study found no evidence of benefit for *L. rhamnosus* GG compared to placebo for induction of remission (OR 0.80, 95% CI 0.04-17.20), with confidence intervals which were wide and thus, did not exclude the potential for benefit or harm.

Eleven studies of probiotics for maintenance of remission in adults and children with Crohn's disease were identified. The identified studies were heterogeneous in inclusion criteria, the probiotic studied, and study design. The probiotic formulations studied included *Escherichia coli* (*E. coli*)Nissle 1917, *S. boulardii*, *L. rhamnosus* GG, *Lactobacillus johnsonii* LA1, and an 8-strain probiotic combination of *L. casei* + *L. plantarum* + *L. acidophilus* + *L. delbrueckii* subsp. *bulgaricus* + *Bifidobacterium longum* subsp. *longum* + *Bifidobacterium breve* + *Bifidobacterium longum* subsp. *infantis* + *S. salivarius* subsp.

thermophilus. In addition, the studies differed in whether remission was induced by medical or surgical therapy, and only one study enrolled children. Lastly, some of the studies used mesalamine as the comparator, or allowed co-therapy with mesalamine in the probiotic arm. Overall, there was no overall evidence of benefit from any of the probiotic therapies studied for maintenance of remission.

The overall quality in the evidence was rated as low for induction of remission and maintenance of remission. Given the overall small study samples, as well as heterogeneity in patient populations, probiotic strains studied, and study design, it is unclear if there is potential for specific probiotic strains to be beneficial for either induction or maintenance of remission in Crohn's disease. Further studies are needed to define specific populations of patients with Crohn's disease who might benefit from probiotics, as well as the most effective probiotic strains.

In patients with ulcerative colitis, the AGA recommends the use of probiotics only in the context of a clinical trial.

GRADE: *No recommendation, knowledge gap.*

The AGA recommends the use of probiotics in adults and children with ulcerative colitis only in the context of a clinical trial. As with Crohn's disease, interest in microbiota-based therapies for ulcerative colitis is growing. However, available evidence is limited because of heterogeneity in study design, patient populations, and the specific probiotics that have been studied.

The technical review identified eleven studies of the use of probiotics for induction of remission in adults and children with ulcerative colitis. The probiotic formulations under evaluation included the two-strain combination of *B. breve* + *B. bifidum*, *B. longum* subsp. *longum* BB536, *E. coli* Nissle 1917, *L. reuteri* ATCC 55730, and the eight-strain combination of *L. casei* + *L. plantarum* + *L. acidophilus* + *L. delbrueckii*

subsp. *bulgaricus* + *B. longum* subsp. *longum* + *B. breve* + *B. longum* subsp. *infantis* + *S. salivarius* subsp. *thermophilus*. The comparators varied among studies, and in some studies included mesalamine.

Four studies compared the 8-strain probiotic combination to mesalamine or balsalazide for induction of remission, suggesting potential for benefit but with very low certainty of the evidence (RR 1.72, 95% CI 0.78-3.32). Two studies examined the effectiveness of oral *E. coli* Nissle 1917 compared to mesalamine for this indication, again with uncertain benefit (RR 0.86, 95% CI 0.49-1.49). One of these studies also allowed adjunctive treatment with steroids and gentamicin, while the other allowed co-therapy with topical prednisolone. One study of rectally administered *E. coli* Nissle 1917 did not show any clear evidence of benefit compared to placebo. Rectally administered *L. reuteri* ATCC 55730 was tested in children, with suggestion of an increased clinical response rate compared to placebo (RR 1.83, 95% CI 1.14-2.92). Other probiotics were tested only in single studies, with no demonstrated benefit for induction of remission.

The technical review identified six studies of probiotics for maintenance of remission in ulcerative colitis. Two studies of *E. coli* Nissle 1917 and one study of *L. rhamnosus* GG (RR 0.82, 95% CI 0.60-1.11) did not show clear benefit of the probiotic compared to mesalamine for maintenance of remission. In addition, compared to placebo, the two-strain combination of *L. acidophilus* LA-5 + *B. animalis* subsp. *lactis* BB12, the two-strain combination of *B. breve* Yakult + *L. acidophilus*, and the three-strain combination of *Streptococcus faecalis* T-111 + *Clostridium butyricum* TO-A + *Bacillus mesentericus* TO-A did not show any evidence of benefit for this indication, although these formulations were only tested in single studies.

The overall quality of evidence for probiotics for induction or maintenance of remission in ulcerative colitis was rated as low. Available evidence is limited by small sample sizes, differences in patient populations, variability in study design, and heterogeneity in the probiotic formulations used. The most

extensively tested formulation was the eight-strain combination of *L. casei* + *L. plantarum* + *L. acidophilus* + *L. delbrueckii* subsp. *bulgaricus* + *B. longum* subsp. *longum* + *B. breve* + *B. longum* subsp. *infantis* + *S. salivarius* subsp. *thermophilus* for induction of remission, although even here the available studies were limited by potential for bias and pooled results did not show evidence of benefit. Further research is needed to identify specific patient populations that might benefit most from treatment with probiotics and to define the most effective probiotic formulations.

In patients with pouchitis, the AGA suggests the use of the eight-strain combination of *L.actobacillus casei* + *Lactobacillus plantarum* + *Lactobacillus acidophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus* + *Bifidobacterium longum* + *Bifidobacterium breve* + *Bifidobacterium infantis* + *Streptococcus salivarius* subsp. *Thermophilus* over no or other probiotics.

GRADE: *Conditional recommendation, very low quality of evidence*

Comment: Patients for whom the feasibility and cost of using this combination of bacterial strain is problematic may reasonably select no probiotics.

The AGA suggests the use of the eight-strain combination of *L. casei* + *L. plantarum* + *L. acidophilus* + *L. delbrueckii* subsp. *bulgaricus* + *B. longum* subsp. *longum* + *B. breve* + *B. longum* subsp. *infantis* + *S. salivarius* subsp. *thermophilus* over no or other probiotics in patients with pouchitis. Pouchitis is a frequent post-surgical complication after total proctocolectomy and ileal pouch-anal anastomosis for ulcerative colitis, and a role for the gut microbiota has been suggested in its pathogenesis. The possibility of microbiota-directed therapy for this condition has been suggested.

The technical review identified seven studies of probiotics for treatment or prevention of pouchitis in adult patients with an ileal pouch-anastomosis for management of ulcerative colitis. The eight-strain probiotic formulation for maintenance of remission in chronic pouchitis was tested in 2 studies including a total of 76 patients, with a potential benefit in the proportion of patients who maintained remission at 12 months compared to placebo (RR 20.24, 95% CI 4.28-95.81, low certainty of evidence). Two additional studies suggested a benefit of the same eight-strain combination for prevention of an initial episode of acute pouchitis, but with very low certainty of evidence (RR for no episodes of pouchitis 1.29, 95% CI 1.03-1.61). Single trials of *L. rhamnosus* GG, *B. longum* subsp. *longum*, and *C. butyricum* MIYAIRI did not show clear evidence of benefit for treatment or prevention of pouchitis, although samples sizes were extremely small in all available studies.

The overall quality of evidence was rated as very low due to risk of bias, small sample sizes, and heterogeneity in the patient populations and interventions tested. The majority of evidence came from studies of the eight-strain probiotic combination of *L. casei* + *L. plantarum* + *L. acidophilus* + *L. delbrueckii* subsp. *bulgaricus* + *B. longum* subsp. *longum* + *B. breve* + *B. longum* subsp. *infantis* + *S. salivarius* subsp. *thermophilus*. Other probiotic formulations need further testing for this indication. It also unclear if these results would apply to children or to patients who underwent an ileal pouch-anal anastomosis for conditions other than chronic ulcerative colitis, such as familial adenomatous polyposis.

In symptomatic children and adults with irritable bowel syndrome, we recommend the use of probiotics only in the context of a clinical trial.

GRADE: *No recommendations, knowledge gap.*

The AGA makes no recommendations for the use of probiotics in children and adults with irritable bowel syndrome (IBS). While there are many studies examining this question, they are marked by significant heterogeneity in both study design, outcome, and probiotics used.

The technical review found a total of 76 RCTs that used 44 different probiotic strains or combinations of strains[tech review ref]. For the majority of studies which reported benefit, the data was derived from a single RCT. Only two probiotics or probiotic combination (*S. boulardii* and the 8-strain combination) had more than one RCT which measured the same outcome allowing for combined analysis. Three studies tested *S. boulardii* in 232 adults with IBS and while the studies used different outcome measures, all reported an abdominal pain score which was not different between those treated with *S. boulardii* vs placebo. Two RCTs tested the 8-strain combination (*L. casei, L. plantarum, L. acidophilus, L. delbrueckii* subsp. *bulgaricus, B. longum* subsp. *longum, B. breve, B. longum* subsp. *infantis*, and *S. salivarius* subsp. *thermophilus)* in 73 adults with IBS and abdominal pain and while this demonstrated a decrease in the abdominal pain score using the visual analogue scale (Mean decrease 3.78: 95% CI 4.93-2.62), the overall sample size was small and there was unclear risk of selection, reporting and detection bias. In addition, the patients enrolled were of variable IBS subtype.

In the remainder of the studies, the majority of the single RCT's using different probiotic and probiotic combinations of variable duration reported some benefit but the sample sizes were all relatively small and had significant differences in study subjects and design. The overall quality of evidence was very low. There also significant concern for publication bias as the technical review team found numerous registered protocols that yielded no peer reviewed publications or results that were publicly available. Thus, while there has been significant interest and potential for the use of probiotics in IBS, further studies are needed to clarify this important question.

In children with acute infectious gastroenteritis, we suggest against the use of probiotics.

GRADE: *Conditional recommendation, moderate quality of evidence*

The AGA suggests against the use of probiotics in children with acute infectious gastroenteritis. The majority of the data supporting the use of probiotics in children with acute infectious gastroenteritis were from studies performed outside of North America while two high qualities studies performed in United States and Canada did not show any benefit.

The technical review identified 89 studies, 58 were included in a Cochrane Review published in 2010 and 31 additional studies were published after 2010. Most of the studies which showed a benefit of probiotics were published in India, Italy, Poland, Turkey, and Pakistan and had one or more concerns regarding risk of bias[technical review]. Of the 89 studies, 58 reported duration of diarrhea as an outcome. Combining these 58 studies which utilized various strains of probiotics, the mean duration of diarrhea was reduced by 21.91 hours (95% CI, 16.17-27.64) but the level of evidence was low. The most commonly studied probiotic was *S. boulardii* which was utilized in 21 RCTs. Only 9 studies reported on the mean duration of diarrhea which was reduced by 28.9 hours (95% CI 16.78-41.03) but the level of evidence was very low. The second most commonly used strain was *L. rhamnosus* GG which was evaluated in 19 RCTs. Of these 19 RCTs, 14 studies reported mean duration of diarrhea as an outcome which was reduced by 23.13 hours (95% CI 12.33-33.94).

While some strains of bacteria improved diarrhea duration in children, few of the studies were performed in North America until two recent multicenter, randomized, double-blind, placebo-controlled trials conducted by the Pediatric Emergency Care Applied Research Network and the Pediatric Emergency Research Canada. These studies enrolled 943 and 827 children from 10 and 6 emergency departments in

the United States and Canada, respectively. The U.S. study used *L. rhamnosus* GG and the Canadian study used a combination of *L. rhamnosus* R0011 and *Lactobacillus helveticus* R0052 for 5 days. Neither showed any benefit in the occurrence of moderate-to-severe gastroenteritis between placebo and probiotics groups. Two additional studies in the United States and Canada using the same strains of bacteria confirmed the lack of benefit. Given likely differences in host genetics, diet, sanitation, and endemic enteropathogens between North America and the other global regions as well as different causes of acute infectious gastroenteritis in children, we do not feel that the studies conducted in other regions can be generalized to the population served by the AGA and thus suggest against the use of probiotics for acute infectious gastroenteritis in children.

In preterm infants (less than 36 weeks gestational age), we suggest using *Lactobacillus* spp. and *Bifidobacterium* spp. or *Bifidobacterium. animalis* subsp. *lactis* or *Lactobacillus. reuteri* over no and other probiotics.

GRADE: *Conditional recommendation, moderate/high quality of evidence*

The AGA suggests the use of certain probiotic strain or strain combination for the prevention of necrotizing enterocolitis (NEC) in preterm infants less than 36 weeks gestational age. Preterm birth is common, affecting 10% of newborns in the United States and 15 million pregnancies worldwide each year. Premature infants have increased risk of mortality and multiple morbidities, including NEC. NEC is the most important gastrointestinal emergency among preterm neonates, characterized by mucosal or even deeper intestinal necrosis of the bowel with common long-term sequelae including short bowel syndrome and impaired neurodevelopment. Microbiota differs in infants with NEC compared to healthy infants providing a rationale for microbiota-oriented treatments.

The technical review presented results from a recent systematic review and network meta-analysis [Add reference when available] that assessed the role of probiotics in the prevention of mortality and morbidity in preterm infants. In total, 63 studies comparing single- and multiple-strain probiotics to placebo in patients with severe NEC were included and multiple outcomes, such as all-cause mortality, severe NEC (stage II or greater), culture-proven sepsis, and duration of hospitalization were assessed.

Single strains or two-strain combinations of *Lactobacillus* spp. and *Bifidobacterium* spp. reduced all-cause mortality compared to placebo (OR: 0.56, 95% CI: 0.39-0.80), while severe NEC was reduced by two-strain combinations of a *Lactobacillus* sp. and a *Bifidobacterium* sp. (OR: 0.35; 95% CI: 0.20, 0.59), *B. animalis* subsp. *lactis* (OR: 0.31; 95% CI: 0.13, 0.74) and *L. reuteri* (OR: 0.55; 95% CI: 0.34, 0.91), all supported by moderate- or high-quality evidence.

There was low to very-low quality of evidence to support beneficial effects of three strain combinations of *Lactobacillus* sp., a *Bifidobacterium* sp., and an *Enterococcus* sp. (OR: 0.28; 95% CI: 0.16, 0.49), two-strain combinations of a *Bifidobacterium* sp. and *S. salivarius* subsp. *thermophilus* (OR: 0.38; 95% CI: 0.19, 0.75) or a *Bacillus* sp. and an *Enterococcus* sp. (OR: 0.23; 95% CI: 0.08, 0.63) in severe NEC reduction compared to placebo.

Three-strain combinations of a *Lactobacillus* sp., a *Bifidobacterium* sp., and *S. boulardii* reduced days to reach full enteral feeds (MD: -3.30; 95% CI: -5.91, -0.69), supported by moderate- or high-quality evidence. Similar effects, although based on low- or very-low quality of evidence was shown with two-strain combinations of a *Lactobacillus* sp. and a *Bifidobacterium* sp. (MD: -2.15; 95% CI: -3.78, -0.51), or with *L. reuteri* (MD: -2.62; 95% CI: -4.53, -0.71). Finally, *B. animalis* subps. *lactis* and *L. reuteri* significantly shortened hospitalization based on moderate- or high-quality evidence (MD: -13.00; 95% CI: -22.71, -3.29 and MD: -7.89, 95% CI: -11.60, -4.17, respectively).

Given the moderate-to-high quality evidence for reduction in all-cause mortality, severe NEC, days to reach full enteral feeding, and days of hospitalization, we suggest using the two-genus combination of *Lactobacillus* spp. and *Bifidobacterium* spp., *B. animalis* subsp. *lactis*, or *L. reuteri* over placebo or other probiotics in premature infants.

Summary

The gut microbiome plays an important role in gastrointestinal health and disease and probiotics represent a promising modality for therapeutic intervention. The current evidence suggests that the use of certain probiotic strains or probiotic strain combinations may prevent *C. difficile* infections for adults and children on antibiotic treatment. However, the quality of evidence was low and the reporting of potential harms was not always consistent. Thus, for patients who place a high value on avoidance of potential harms, particularly those with severe illnesses or immunosuppression, it would be reasonable to select not to use probiotics. While there was evidence for probiotics in the prevention of *C. difficile*, the technical review found significant knowledge gap in the use of probiotics in treatment of *C. difficile* and recommend this as an area for further study. Similar knowledge gaps exist in the use of probiotics in irritable bowel disease and inflammatory bowel disease (Crohn's disease and ulcerative colitis). In the subset of patients with pouchitis, current evidence supports the use of the eight-strain combination (*L. casei, L. plantarum, L. acidophilus, L. debrueckii* subsp. *bulgaricus*, *B. longum* subsp. *longum*, *B. breve, B. longum* subsp. *infantis*, and *S. salivarius* subsp. *thermophilus*) if feasibility of obtaining the combination is not an issue. In preterm infants less than 36 weeks gestational age, the probiotic strains (*B. animalis* subsp. *lactis* or *L. reuteri)* or combination of 2 strains (*Lactobacillus* spp. and *Bifidobacterium* spp.) may prevent the development of NEC. For children with acute gastroenteritis in North America, however, the current evidence does not support the use of probiotics.

We identified that significant knowledge gaps exist in this very promising and important area of research due to the significant heterogeneity between studies and variability in the probiotic strains studied. The lack of consistent harms reporting make it difficult to assess true harms. The lack of product manufacturing details prohibits true comparisons and decreases the feasibility of obtaining certain products by patients. Future high-quality studies are urgently needed which address these pitfalls.

Table 1: Quality of Evidence

High	We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate	We are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low	Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect.
Very Low	We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect

Table 2: Strength of Recommendation

	For the Patient	*For the Clinician*
Strong	Most individuals in this situation would want the recommended course of action and only a small proportion would not.	Most individuals should receive the recommended course of action. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences.
Conditional	The majority of individuals in this situation would want the suggested course of action, but many would not.	Different choices will be appropriate for different patients. Decision aids may be useful in helping individuals in making decisions consistent with their values and preferences. Clinicians should expect to spend more time with patients when working towards a decision.

Table 3: Summary of recommendations:

Recommendations	Strength of Recommendation	Quality of Evidence
1. In patients with *C. difficile* infection, we recommend the use of probiotics only in the context of a clinical trial.	No recommendation	Knowledge gap
2. In adults and children on antibiotic treatment, we suggest the use of *S. boulardii*; or the two-strain combination of *L. acidophilus* and *L. casei*; or the three-strain combination of *L. acidophilus*, *L. delbruekii* subsp. *bulgaricus*, and *B. bifidum*; or the four-strain combination of *L. acidophilus, L. delbruekii* subsp. *bulgaricus*, *B. bifidum*, and *S. thermophilus* over no or other probiotics for prevention of *C. difficile* infection. Comment: Patients who place a high value on the potential harms (particularly those with severe illnesses) or a high value on avoiding the associated cost and a low value on the small risk of C. *difficile* development (particularly in the outpatient setting), would reasonably select no probiotics.	Conditional	Low
3. In patients with Crohn's disease, we recommend the use of probiotics only in the context of a clinical trial.	No recommendation	Knowledge gap
4. In patients with ulcerative colitis, we recommend the use of probiotics only in the context of a clinical trial.	No recommendation	Knowledge gap
5. In patients with pouchitis, we suggest the use of *L. casei, L. plantarum, L. acidophilus, L. debrueckii* subsp. *bulgaricus, B. longum* subsp. *longum, B. breve, B. longum* subsp. *infantis*, and *S. salivarius* subsp. *thermophilus* over no or other probiotics. Comment: Patients for whom the feasibility and cost of using this combination of bacterial strain is problematic may reasonably select no probiotics.	Conditional	Very low
6. In symptomatic children and adults with irritable bowel syndrome, we recommend the use of probiotics only in the context of a clinical trial.	No recommendations	Knowledge gap
7. In children with acute infectious gastroenteritis, we suggest against the use of probiotics.	Conditional	Moderate
8. In preterm infants (less than 36 weeks GA), we suggest using *Lactobacillus* spp. and *Bifidobacterium* spp. or *B. lactis* or *L. reuteri* for prevention of NEC over no and other probiotics.	Conditional	Moderate/high

References

1. Sanders ME, Merenstein DJ, Reid G, et al. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol 2019;16:605-616.
2. Suez J, Zmora N, Segal E, et al. The pros, cons, and many unknowns of probiotics. Nat Med 2019;25:716-729.
3. Sniffen JC, McFarland LV, Evans CT, et al. Choosing an appropriate probiotic product for your patient: An evidence-based practical guide. PLoS One 2018;13:e0209205.
4. Caselli M, Cassol F, Calo G, et al. Actual concept of "probiotics": is it more functional to science or business? World J Gastroenterol 2013;19:1527-40.
5. de Simone C. The Unregulated Probiotic Market. Clin Gastroenterol Hepatol 2019;17:809-817.
6. Bindels LB, Delzenne NM, Cani PD, et al. Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 2015;12:303-10.
7. Parker EA, Roy T, D'Adamo CR, et al. Probiotics and gastrointestinal conditions: An overview of evidence from the Cochrane Collaboration. Nutrition 2018;45:125-134 e11.
8. Clarke TC, Black LI, Stussman BJ, et al. Trends in the use of complementary health approaches among adults: United States, 2002-2012. Natl Health Stat Report 2015:1-16.
9. Sultan S, Falck-Ytter Y, Inadomi JM. The AGA institute process for developing clinical practice guidelines part one: grading the evidence. Clin Gastroenterol Hepatol 2013;11:329-32.
10. Graham R, Mancher M, Dianne W, et al. Clinical Practice Guidelines We Can Trust. Washinton DC: National Academies Press, 2011.
11. Lessa FC, Mu Y, Bamberg WM, et al. Burden of Clostridium difficile infection in the United States. N Engl J Med 2015;372:825-34.
12. Vaughn BP, Rank KM, Khoruts A. Fecal Microbiota Transplantation: Current Status in Treatment of GI and Liver Disease. Clin Gastroenterol Hepatol 2019;17:353-361.
13. Goldenberg JZ, Yap C, Lytvyn L, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 2017;12:CD006095.