Effect of Probiotics on Inducing Remission and Maintaining Therapy in Ulcerative Colitis, Crohn's Disease, and Pouchitis: Meta-analysis of Randomized Controlled Trials

Jun Shen, MD, PhD, Zhi-Xiang Zuo, PhD,† and Ai-Ping Mao, PhD‡*

Background: Whether probiotics are beneficial at all stages of treatment in inflammatory bowel disease or superior to placebo remains controversial.

Methods: Two reviewers independently selected randomized controlled trials comparing probiotics with controls in inflammatory bowel disease and extracted data related to remission/response rates, relapse rates, and adverse events. Subanalyses were also performed.

Results: Twenty-three randomized controlled trials with a total of 1763 participants met the inclusion criteria. From the meta-analysis, probiotics significantly increase the remission rates in patients with active ulcerative colitis (UC) ($P = 0.01$, risk ratio [RR] = 1.51). The remission rates were significantly higher in patients with active UC treated with probiotics than placebo ($P < 0.0001$, RR = 1.80). Unfortunately, subgroup analysis found that only *VSL#3* significantly increased the remission rates compared with controls in patients with active UC ($P = 0.004$, RR = 1.74). Interestingly, *VSL#3* ($P < 0.00001$, RR = 0.18) also significantly reduced the clinical relapse rates for maintaining remission in patients with pouchitis. No significantly different adverse events were detected between probiotics and controls in the treatment of UC ($P = 0.94$, RR = 0.99) or CD ($P = 0.33$, RR = 0.87).

Conclusions: Administration of probiotics results in additional benefit in inducing remission of patients with UC. *VSL#3* are beneficial for maintaining remission in patients with pouchitis. And, probiotics can provide the similar effect as 5-aminosalicylic acid on maintaining remission of UC, although no additional adverse events presented.

(Inflamm Bowel Dis 2014;20:21–35)

Key Words: probiotics, remission, relapse, pouchitis, inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing disorder. There is a growing body of evidence on the connection between intestinal microflora and pathogenesis of IBD. The intestinal flora has a conditioning effect on intestinal homeostasis, delivering regulatory signals to the epithelium, the mucosal immune system, and the neuromuscular activity of the gut.[1,2] Clinical and experimental studies suggest that the relative balance of aggressive and protective bacterial species is altered in ulcerative colitis (UC), Crohn's disease (CD), and pouchitis, which are caused by overly aggressive immune responses to a subset of commensal enteric bacteria in genetically predisposed individuals.[3] Because intestinal microflora play a pivotal role in the development of IBD, there is currently some interest in altering the composition of the microflora toward a potentially more remedial community.[4] Probiotics are live and nonpathogenic bacteria that confer health benefits beyond their nutritional value. In IBD, where changes in bacterial flora have been demonstrated, there is an increasing interest in modulating the flora with probiotic strains.[5]

Researchers have suggested that probiotics might offer an alternative or adjuvant approach to conventional therapy by altering the intestinal microflora and modulating the host immune system.[6] In vitro and animal studies have shown that probiotics influence the levels of inflammatory cytokines and amelioration of the production of some mediators involved in the inflammatory response of the intestine and gut permeability.[7,8] Therefore, studies on appropriate animal models have better clarified our knowledge about probiotic bacteria, and the potential application of probiotics as a valid therapeutic option in patients with IBD.

The induction and maintenance of disease remission and prevention of complications, such as pouchitis, are primary goals in the management of IBD.[9] Alterations of the bacterial microbiota may be an important factor that triggers the disease process.[10,11] Remission periods are often short, and the conditions are more

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.ibdjournal.org).

Received for publication September 16, 2013; Accepted October 15, 2013.

From the *Department of Gastroenterology, Renji Hospital, Shanghai Jiao-Tong University, School of Medicine, Shanghai Institute of Digestive Disease, Shanghai, China; †Department of Medicine, University of Chicago, Chicago, Illinois; and ‡Committee on Immunology, Department of Pathology, University of Chicago, Chicago, Illinois.

Supported by grants from National Natural Science Foundation of China (No. 81000161 and No.81170362).

The authors have no conflicts of interest to disclose.

Reprints: Ai-Ping Mao, PhD, Committee on Immunology, Department of Pathology, University of Chicago, Chicago, IL 60637 (e-mail: amao@bsd.uchicago.edu).

DOI 10.1097/01.MIB.0000437495.30052.be

Published online 25 November 2013.

complicated after frequent relapses. Pouchitis, a common complication of ileal pouchanal anastomosis surgery for UC, is a nonspecific inflammation of the ileal reservoir with reduced counts of *Lactobacilli* and *Bifidobacteria* within the pouch.[12] Probiotic mechanisms of action have led to new support for the use of probiotics in the management of IBD. However, intervention trials using probiotics have provided conflicting evidence. Earlier reviews for IBD were not able to make definitive conclusions about the value of probiotics for IBD.[13] Furthermore, it is still controversial whether probiotics are beneficial at all stages of treatment and superior to placebo.

Although several randomized clinical trials (RCTs) suggested that specific probiotic was efficacious for the induction and maintenance of remission in UC, CD, or pouchitis, only limited results extracted from Cochrane meta-analyses showed that there was insufficient evidence to make clear conclusions about the efficacy of probiotics for the induction or maintenance of remission in UC or CD until the year 2011.[14,15] Thus, we carried out meta-analysis of RCTs to comprehensively evaluate the effect and adverse events of probiotics in patients with IBD, with special focus on UC, CD, and pouchitis.

METHODS

Search Strategy

We searched for all RCTs by using MEDLINE (1966 to March, 2013), EMBASE (1980 to March, 2013), the Cochrane Controlled Trials Register (first Quarter, 2013), OVID (1950 to March, 2013), BIOSIS (1996 to December, 2012), and the Chinese Biomedical Database (1981 to December, 2012) to identify comparative studies of probiotics in IBD. The following keywords were used in combinations of the search: "probiotic," "inflammatory bowel disease," "ulcerative colitis," "Crohn's disease," "pouchitis," "*Lactobacillus*," "*Bifidobacterium*," "*Saccharomyces*," "*Escherichia coli*," and "*VSL#3*." A comprehensive search of reference lists of all review articles and original studies retrieved by this method was performed to identify additional reports. Furthermore, we hand searched abstracts of major gastroenterological meetings, such as the Digestive Disease Week of the American Gastroenterological Association and the World Congress of Gastroenterology. No language restrictions were made. Authors of some identified trials were asked whether they knew of additional studies, including unpublished randomized ones. We scanned the titles and the abstracts of the trials to exclude studies that were considered irrelevant. Then, we identified trials that fulfilled the inclusion criteria from full texts of the remaining studies.

Selection Criteria

The selection criteria were as follows:

We included RCTs. Studies were not included if they did not provide details on the patient selection, allocation, study design, outcomes, or measurement methods. Studies in abstract form or meeting report, without publication of the full article, were also included in the analyses.

Both adult patients and children were included in the analyses.

Studies included at least 2 branches: control group received aminosalicylates, steroids, or/and azathioprine with/without placebo when introduction of remission and placebo with/without aminosalicylates, or only 5-aminosalicylic acid (5-ASA) when maintenance of remission; probiotics group received probiotics, or probiotics plus the same control treatment.

Articles were included if they provided information on at least 1 outcome parameter as follows: the remission/response rate, the relapse rate, the clinical disease activity index, the endoscopic assessment, the histologic assessment, the adverse events, and the withdrawals.

Furthermore, articles published in English or other languages were also included if a translation was provided by the authors.

Data Extraction

We prepared standardized data abstraction sheets before data extraction. All the data were tabulated. We extracted from data for author, year, location of trials, trial design, disease of patients, duration of interventions, number of participants, details of interventions, measurements of outcomes, and study quality. All papers were examined independently for eligibility by 2 reviewers (J.S. and Z.-X.Z.). Disagreements were resolved by consulting a third reviewer (A-P.M.). Study quality was assessed using the Jadad score system based on 5 items: was the study described as randomized; was the method used to generate the sequence of randomization described and appropriate; was the study described as double blind; was the method of double blinding described and appropriate; was there a description of withdrawals and dropouts?[16] The range of possible scores is 0 to 5. The selected studies were scored independently by 2 investigators (J.S. and Z-X.Z.), and if there were disagreements, then they discussed or consulted a third reviewer to obtain the final scores (A-P.M.). We excluded the trials with Jadad scores <4, and the excluding reasons are listed in Table, Supplemental Digital Content 1, http://links.lww.com/IBD/A350.

Statistical Analysis

We tested dichotomous data by calculating the rate difference with their 95% confidence interval. We used random effects models to examine the risk ratios by intention to treat and considered $P \leq 0.05$ (2 sided) as significant. Heterogeneity within the studies was assessed using chi-square test. Statistical significance for the test of heterogeneity was set at 0.10.

Subgroup analyses for the meta-analyses were planned depending on diseases, study designs, and species of probiotics. We performed sensitivity analyses by estimating the risk ratios in the absence of 1 or more studies to evaluate the stability and reduced the heterogeneity of the results of our meta-analyses. Statistical analysis was conducted with the software Review Manager 5.0 (Cochrane Collaboration, Oxford, United Kingdom).

RESULTS

We identified 4104 citations and abstracts obtained from literature searches. The 44 potentially eligible studies were obtained for further assessment after screening of titles and abstracts. Twenty-three randomized controlled trials were identified to meet the inclusion criteria.[17–39]

Study Characteristics

The 23 randomized controlled trials enrolled a total of 1763 participants published during 1997 to 2011 (Table 1). Twelve studies presented results for UC[18,19,23,24,27,29,31,33–36,38], 4 for pouchitis,[20,22,25,39] and 7 for CD.[17,21,26,28,30,32,37] The length of follow-up of these trials ranged from 1 to 24 months. Seven trials evaluated remission rates or response rates,[23,27,29,34–36,38] 11 trials evaluated relapse rates,[18,20–22,24,25,28,30–32,39] and 5 evaluated both.[17,19,26,33,37] Three trials evaluated endoscopic relapse rate.[21,30,32] In 1 trial, patients received enema containing *E. coli* (*E. coli*) Nissle 1917 instead of oral administration respectively.[35] Three studies were conducted in children[21,28,33] and 1 was 3 armed.[31] To avoid double counting of the control patients, the results for the arm were shared in subgroup analysis.[31]

All included studies have Jadas scores over 3. In pooled RCTs, 19 studies were double-blind[17–22,24–26,28–30,32–37,39] and 19 were placebo-controlled.[17,18,20–23,25,26,28–30,32–39]

Effect of Probiotics for Inducing Remission/Response in Active Ulcerative Colitis and Crohn's Disease

Effect for Inducing Remission/Response in Active Ulcerative Colitis and Crohn's Disease

Twelve trials reported on the remission or improvement of IBD.[17,19,23,26,27,29,33–38] Data were available for 723 patients, of who 368 received probiotics as supplement treatment and 355 received conventional treatment including salazosulphapyridine, 5-ASA, corticosteroids, and immunosuppressants with or without placebo. The pooled risk ratio (RR) for the remission/response rates of probiotics supplementation versus control group was 1.28, with 95% confidence interval, 1.00 to 1.64. Although meta-analysis showed better effect on probiotics supplementation for IBD ($P = 0.05$), remission/response rates from these 12 trials still showed some heterogeneity ($P = 0.001$, $I^2 = 64\%$) (Fig. 1). Because of the heterogeneity within the pooled trials, subanalyses according to different diseases are needed. Nine trials included patients with UC[19,23,27,29,33–36,38] and 3 included patients with CD.[17,26,37] Subgroup analyses suggested a significant benefit in favor of probiotics supplement in UC subgroup ($P = 0.01$, RR = 1.51), but not in CD subgroup ($P = 0.35$, RR = 0.89) (Fig. 1). However, we could not ignore that there was significant heterogeneity in UC subgroup ($P = 0.004$, $I^2 = 65\%$).

Sensitivity Analyses for Inducing Remission/Response in Active Ulcerative Colitis and Crohn's Disease

All 12 trials gave information about remission/response rates. However, in 2 trials, control groups received balsalazide and/or mesalazine as control instead of placebo.[19,27] Thus, we excluded these 2 trials to reduce heterogeneity. Fortunately, all the 3 CD trials used placebo as controls,[17,26,37] and there was no significant benefit favoring probiotics supplement ($P = 0.35$, RR = 0.89) with no significant heterogeneity ($P = 0.74$, $I^2 = 0\%$) (Fig. 2). Sensitivity analyses decreased the heterogeneity in UC subgroup ($P = 0.39$, $I^2 = 4\%$) and suggested that probiotic supplements had significantly better effect compared with placebo for remission/response rates ($P < 0.0001$, RR = 1.80) (Fig. 2).

Rate of Remission/Response in Ulcerative Colitis with Different Probiotic Supplements

Because different probiotics were used for remission/response in UC, we divided the 9 trials into 3 subgroups: *Bifidobacteria*,[23,29] *E. Coli*,[19,35] and *VSL#3*.[27,33,34,36,38] Meta-analyses suggested that only *VSL#3* significantly increased the rate of remission/response ($P = 0.004$, RR = 1.74) (Fig. 3). The other 3 subgroups showed no statistically significant difference between probiotic group and controls, which suggested that combined probiotics provide better clinical efficacy. Since only 3 trials were included on CD, and *E. Coli*,[17] *Lactobacillus rhamnosus* strain GG,[26] and *Bifidobacteria*[37] were applied, respectively, meta-analyses for remission/response in CD with different probiotic supplements were not applicable.

Effect of Probiotics for Maintaining Therapy in Ulcerative Colitis, Crohn's Disease, and Pouchitis

Clinical Relapse in Maintaining Therapy in Ulcerative Colitis, Crohn's Disease, and Pouchitis

To date, maintaining remission is still one of the goals in IBD therapy. Therefore, a total of 16 trials[17–22,24–26,28,30–33,37,39] that reported clinical relapse rates were combined in the analysis of maintaining remission. Data were available for 1208 patients, of whom 637 received probiotics as maintaining treatment and 571 received controls with the follow-up ranged from 2 to 42 months. Significant benefit in favor of probiotics administration for lower relapse rates ($P = 0.04$, RR = 0.73) was indicated in the meta-analysis (Fig. 4). However, there was moderate to high heterogeneity among these studies ($P = 0.001$, $I^2 = 59\%$).

Subanalyses for Clinical Relapse in Maintaining Therapy in Ulcerative Colitis, Crohn's Disease, and Pouchitis

Pooled estimates were, however, characterized by considerable heterogeneity in the meta-analysis of overall clinical relapse rates (Fig. 4). Subgroup analyses were carried out on the criteria

TABLE 1. Characteristics of Included Studies in this Meta-analysis

Trial	Study Design	Patients and Duration	Number of Patients Analyzed (Probiotics/Control)	Probiotics Administration	Intervention of Control Group	Outcome Definitions	Outcome Extracted (Probiotics/Control)
Malchow[17] 1997, Germany	Single-center RCT: double-blind: placebo-controlled	CD: 12 mo	28 (16/12)	Induction of remission: *E. coli* strain Nissle 1917	Induction of remission: prednisolone plus placebo	Remission was defined as a CDAI <150	Remission: (12/11)
				Maintenance of remission: *E. coli* strain Nissle 1917	Maintenance of remission: placebo		Relapse (4/7)
Kruis[18]: 1997, Germany, Czech Republic, and Austria	Multicenter RCT: double-blind: Placebo-controlled	UC: 3 mo	103 (50/53)	Maintenance of remission: *E. coli* strain Nissle 1917 plus 5-ASA	Maintenance of remission: 5-ASA plus placebo	Relapse was defined as CAI >6 or an increase in CAI of at least 3 points with CAI = 4 being exceeded at the same time; EI >4; and histological signs of acute inflammation	Relapse: (8/6)
Rembacken[19]: 1999, United Kingdom	Single-center RCT: double-blind	UC: 3 mo	116 (57/59)	Induction of remission: *E. coli* Nissle 1917 and hydrocortisone acetate enemas and prednisolone	Induction of remission: 5-ASA and hydrocortisone acetate enemas and prednisolone	Remission was defined as general well being with the passage of no more than 3 formed stools per day, a rectal mucosa without erythema, granularity, or friability, and histologically inactive disease	Remission: (39/44)
				Maintenance of remission: E coli and hydrocortisone acetate enema and prednisolone	Maintenance of remission: 5-ASA and hydrocortisone acetate enema and prednisolone		Relapse: (26/32)
Gionchetti[20]: 2000, Italy	Single-center RCT: double-blind: placebo-controlled	Pouchitis (UC): 9 mo	40 (20/20)	Maintenance of remission: VSL#3	Maintenance of remission: placebo	Relapse was defined as an increase of at least 2 points in the clinical portion of PDAI	Relapse: (3/20)
Prantera[21]: 2002, Italy	Single-center RCT: double-blind placebo-controlled	CD: 12 mo	32 (15/17)	Maintenance of remission: LGG	Maintenance of remission: Placebo	Relapse was defined as an increase in CDAI >150 points, confirmed by endoscopic signs (endoscopic scoring system of Rutgeerts) of inflammation	Relapse: (3/2)

TABLE 1 (*Continued*)

Trial	Study Design	Patients and Duration	Number of Patients Analyzed (Probiotics/Control)	Probiotics Administration	Intervention of Control Group	Outcome Definitions	Outcome Extracted (Probiotics/Control)
Gionchetti[22] 2003, Italy	Single-center RCT: double-blind: placebo-controlled	Pouchitis (UC) 12 mo	40 (20/20)	Maintenance of remission: VSL#3	Maintenance of remission: placebo	PDAI ≥7 as acute pouchitis	Relapse (2/8)
Kato[23] 2004, Japan	Single-center RCT placebo-controlled	UC 3 mo	20 (10/10)	Induction of remission: *Bifidobacterium breve, Bifidobacterium bifidum*, and *Lactobacillus acidophillus*, plus SASP or 5-ASA	Induction of remission: placebo plus SASP or 5-ASA	Remission was defined as a decrease in the CAI score of at least 3 points, absence of rectal bleeding, a rectal mucosa without erythema, granularity, or friability, and normal or near-normal sigmoidoscopic findings	Remission: (4/3)
Kruis[24]: 2004, Germany	Multicenter RCT: double-blind	UC: 12 mo	327 (162/165)	Maintenance of remission: *E. coli*	Maintenance of remission: 5-ASA	Relapse was defined as CAI >6 or an increase in CAI of at least 3 points with CAI = 4 being exceeded at the same time; EI >4; and histological signs of acute inflammation	Relapse: (40/38)
Mimura[25]: 2004 United Kingdom	Single-center RCT: double-blind placebo-controlled	Pouchitis (UC) 12 mo	36 (20/16)	Maintenance of remission: VSL#3	Maintenance of remission: placebo	Relapse was defined as clinical PDAI score ≥2 and endoscopic PDAI score ≥3	Relapse: (3/15)
Schultz[26]: 2004, Germany	Single-center RCT: double-blind: placebo-controlled	CD: 6 mo	11 (5/6)	Induction of remission: LGG and corticosteroids	Induction of remission: placebo and corticosteroids	Remission was defined as: freedom from relapse at the 6 months follow-up visit	Remission: (4/5)
				Maintenance of remission: LGG	Maintenance of remission: placebo	Relapse was defined as an increase in CDAI >100	Relapse: (2/3)
Tursi[27]: 2004, Italy	Multicenter RCT	UC: 2 months	90 (30/60)	Induction of remission: VLS#3	Induction of remission: balsalazide or 5-ASA	Response was defined as patient functional assessment ratings of normal bowel movements and absence of rectal bleeding	Response: (24/37)

TABLE 1 (*Continued*)

Trial	Study Design	Patients and Duration	Number of Patients Analyzed (Probiotics/Control)	Probiotics Administration	Intervention of Control Group	Outcome Definitions	Outcome Extracted (Probiotics/Control)
Bousvaros[28]: 2005, United States	Multicenter RCT: double-blind: Placebo-controlled	CD: 24 mo	75 (39/36)	Maintenance of remission: LGG plus aminosalicylates, 6-MP, azathioprine, and corticosteroids	Maintenance of remission: Placebo plus aminosalicylates, 6-MP, azathioprine, and corticosteroids	Relapse was defined as: PCDAI >30 points on any single visit or a PCDAI >15 on any 2 consecutive visits more than 1 week apart; need for corticosteroid or other rescue therapy for active CD; need for surgery or hospitalization for a complication of CD	Relapse: (12/6)
Furrie[29]: 2005, United Kingdom	Single-center RCT: double-blind: placebo-controlled	UC: 1 mo	18 (9/9)	Induction of remission: *Bifidobacterium longum* plus steroids, immunosuppressants, or 5-ASA	Induction of remission: placebo plus steroids, immunosuppressants, or 5-ASA	Remission was defined as CAI improved	Remission: (5/3)
Marteau[30]: 2006, France	Multicenter RCT: double-blind: placebo-controlled	CD: 6 mo	98 (48/50)	Maintenance of remission: lyophilized LA1	Maintenance of remission: placebo	Clinical relapse was defined as a CDAI ≥200; endoscopic relapse defined as grade 1 macroscopic lesions in the ileum or colon	Relapse: (4/3)
Zocco[31]: 2006, Italy	Single-center RCT	UC: 12 mo	187 (127/60)	Maintenance of remission: LGG or LGG plus 5-ASA	Maintenance of remission: 5-ASA	Relapse was defined as appearance of symptoms and/or signs needed additional medical treatment and increase in CAI >4	Relapse: (20/12)
Van Gossum[32]: 2007, Belgium	Multicenter RCT: double-blind placebo-controlled	CD: 3 mo	70 (34/36)	Maintenance of remission: lactobacillus johnsonii, LA1	Maintenance of remission: placebo	Relapse was defined as CDAI >150, with an increase of CDAI >70 or greater from baseline	Relapse: (4/3)
Miele[33]: 2009, Italy	Single-center: double-blind: placebo-controlled	UC: 12 mo	29 (14/15)	Induction of remission: VSL#3 plus oral methylprednisolone	Induction of remission: placebo plus oral methylprednisolone	Remission was defined as LCSI ≤2	Remission: (11/4)
				Maintenance of remission: VSL#3 plus oral 5-ASA	Maintenance of remission: placebo plus oral 5-ASA	Relapse was defined as the occurrence or worsening of symptoms, accompanied by an increase in LCAI >3	Relapse: (3/11)
Sood[34]: 2009, India	Multicenter RCT: double-blind: placebo-controlled	UC: 3 mo	147 (77/70)	Induction of remission: VSL#3	Induction of remission: placebo	Remission was defined as UCDAI ≤2	Remission: (25/7)

TABLE 1 (*Continued*)

Trial	Study Design	Patients and Duration	Number of Patients Analyzed (Probiotics/Control)	Probiotics Administration	Intervention of Control Group	Outcome Definitions	Outcome Extracted (Probiotics/Control)
Matthes[35]: 2010, Germany	Multicenter RCT: double-blind: placebo-controlled	UC: 2 mo	57 (46/11)	Induction of remission: *E. coli* strain Nissle 1917	Induction of remission: placebo	Remission was defined as DAI ≤ 2	Remission: (20/3)
Ng[36]: 2010, United Kingdom	Multicenter: double-blind: placebo-controlled	UC: 2 mo	28 (14/14)	Induction of remission: VSL#3	Induction of remission: placebo	Remission was defined as UCDAI ≤ 2	Remission: (7/5)
Steed[37]: 2010, United Kingdom	Single-center RCT: double-blind: Placebo-controlled	CD: 6 mo	35 (19/16)	Induction of remission: *Bifidobacterium longum*, synergy 1	Induction of remission: placebo	Response was defined as changes in CDAI to <150 or a drop in CDAI of >75 from baseline	Remission: (13/11)
			24 (13/11)	Maintenance of remission: *Bifidobacterium longum*, Synergy 1	Maintenance of remission: placebo	Relapse was defines as an increase in CDAI by 100 points or a score >450	Relapse: (8/5)
Tursi[38]: 2010, Italy	Multicenter RCT: placebo-controlled	UC: 2 mo	144 (71/73)	Induction of remission: VSL#3	Induction of remission: placebo	Remission was defined as UCDAI ≤2	Remission: (31/23)
Wildt[39]: 2011, Denmark	RCT, double-blind: placebo-controlled	Pouchitis (UC) 12 mo	32 (20/12)	Maintenance of remission: bifidobacterium	Maintenance of remission: placebo	Relapse was defined as the presence of 1 or less of 3 criteria: SCCAI ≤4, endoscopically grade 0–1, histologically grade 0–1	Relapse: (15/11)

5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; CAI, clinical activity index; CDAI, Crohn's disease activity index; EcN, *E. coli* nissle 1917; EI, endoscopic index; LGG, *Lactobacillus rhamnosus* strain GG; PCDAI, pediatric CD activity index; PDAI, pouchitis disease activity index; SASP, salazosulphapyridine; SCCAI, simple clinical colitis activity index; RCT, randomized controlled trial.

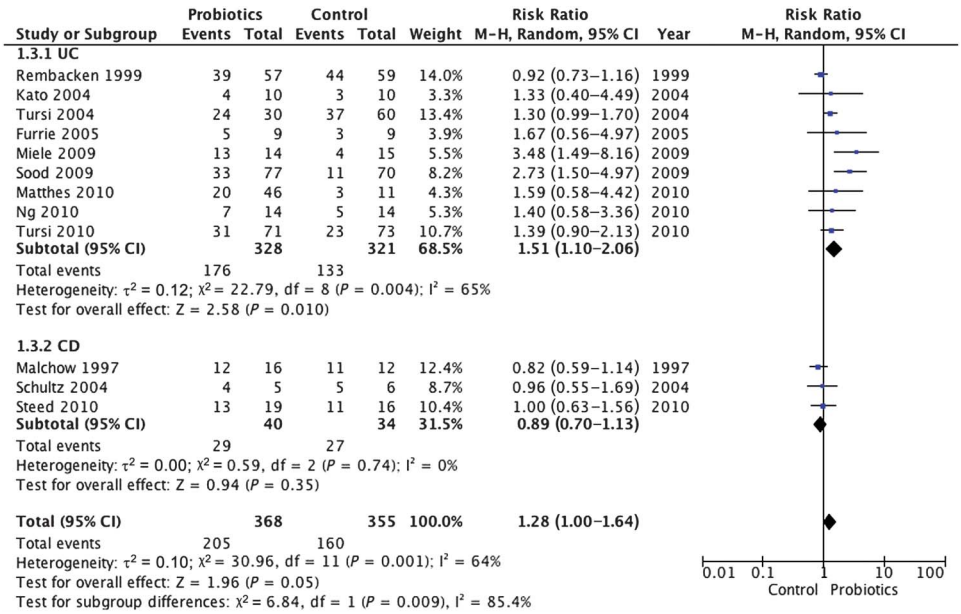

FIGURE 1. The forest plot of the remission/response rates for probiotics compared with control groups in inducing remission of IBD.

specified in the protocol to attempt to explain and reduce such heterogeneity.

We divided pooled trials into 3 subgroups according to different disease types (Fig. 4). Subgroup analysis of 5 studies[18,19,24,31,33] in patients with UC did not show significant advantage in maintaining treatment with probiotics compared with control group ($P = 0.47$, RR = 0.89), and heterogeneity was not significant ($P = 0.19$, $I^2 = 35\%$). Subgroup analysis of 4 trials[20,22,25,39] reported on patients with pouchitis did not show significant benefit in favor of probiotics administration ($P = 0.10$, RR = 0.28). Similarly, when 7 studies[17,21,26,28,30,32,37] recruiting patients with CD were considered in subgroup analysis, no significant difference was found between the interventions ($P = 0.71$, RR = 1.09).

Endoscopic Relapse in Maintaining Therapy in Crohn's Disease

Only 3 trials reported endoscopic relapse rates.[21,30,32] Fortunately, all of the participants pooled in these 3 studies were patients with CD (Fig. 5). No statistically significant advantage was found for patients using probiotics for maintaining treatment ($P = 0.75$, RR = 1.08).

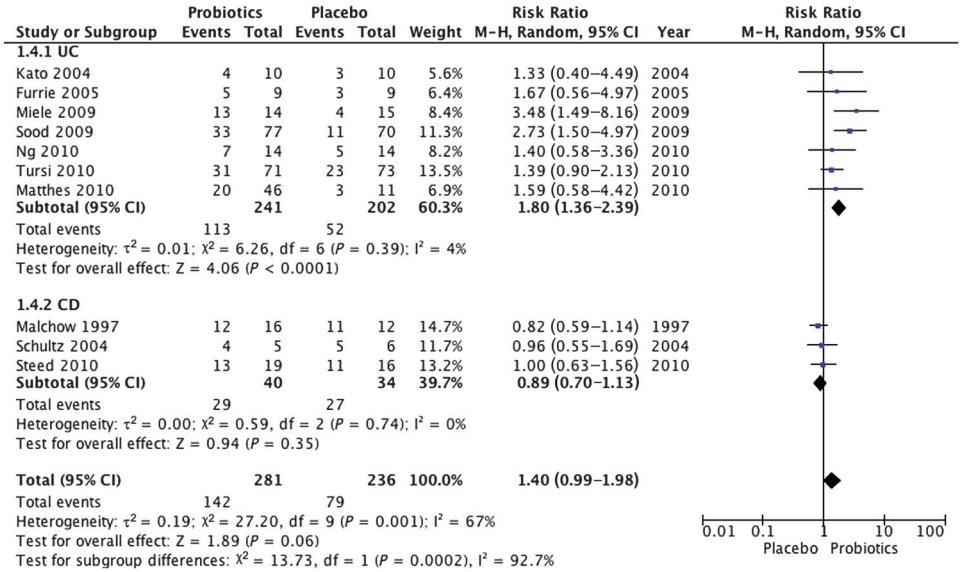

FIGURE 2. The subgroup analysis for the remission/response rates of probiotics compared with placebo in inducing remission of UC and CD.

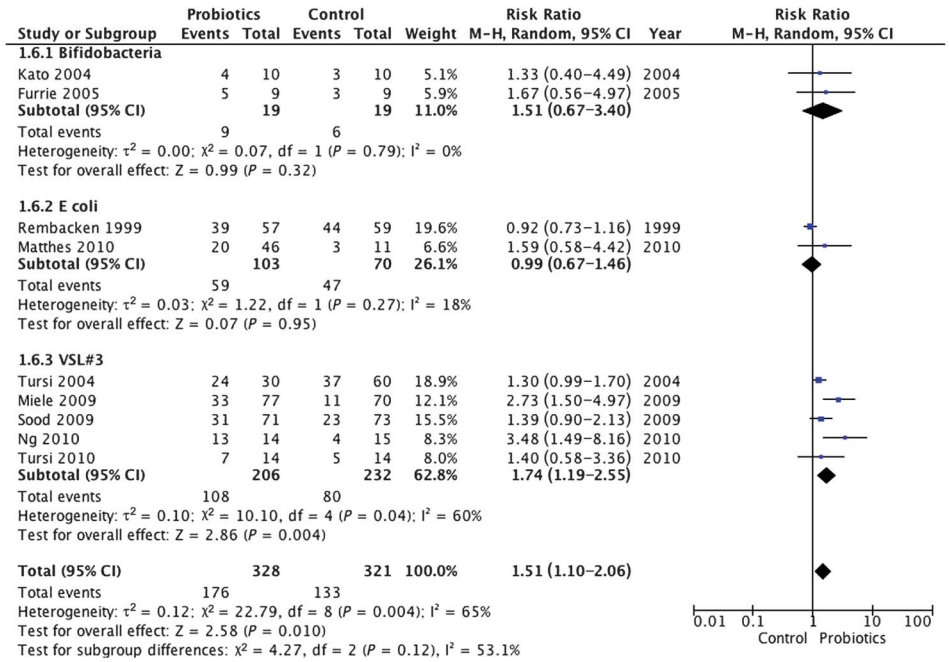

FIGURE 3. The subgroup analysis for the remission/response rates of different probiotics in inducing remission of UC.

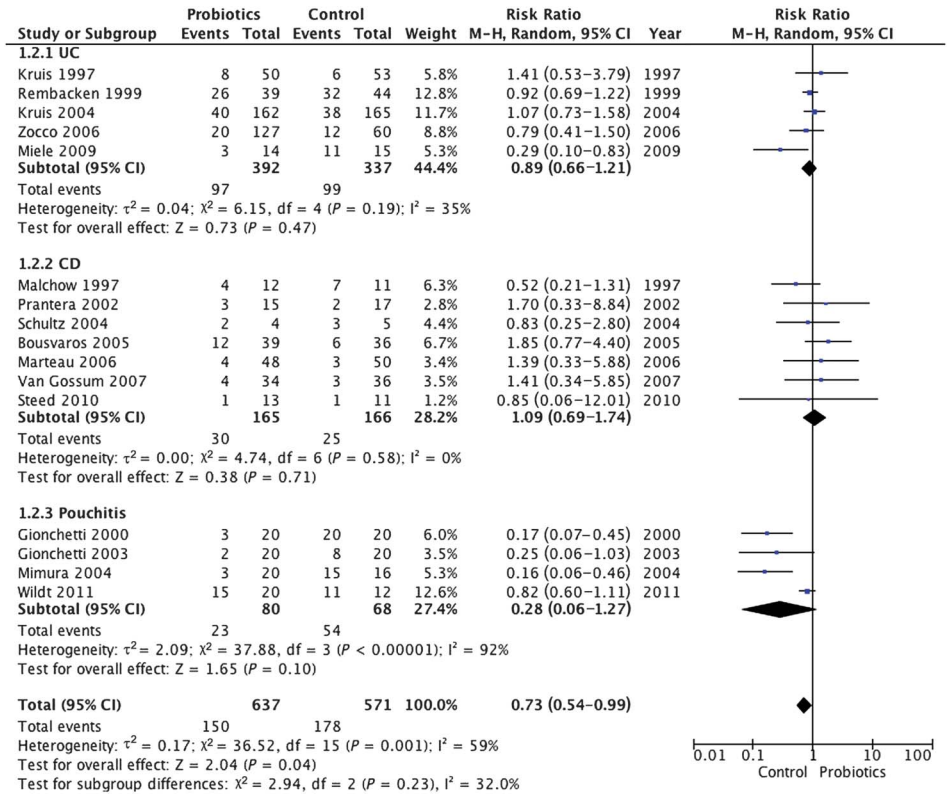

FIGURE 4. The forest plot of the clinical relapse rates for probiotics compared with control groups in maintaining remission of IBD.

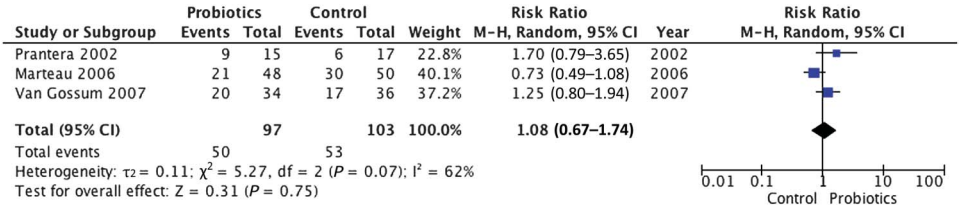

FIGURE 5. The forest plot of the endoscopic relapse rates for probiotics compared with control groups in maintaining remission of CD.

Clinical Relapse in Maintaining Therapy in Ulcerative Colitis, Crohn's Disease, and Pouchitis Based on trial Designs

All the 4 trials[20,22,25,39] in maintaining therapy for pouchitis used placebo as control, and there was no significant difference between probiotics and placebo groups ($P = 0.10$, RR = 0.28) (Fig. 4). We also carried out subgroup analysis on the basis of trial designs for UC[18,19,24,31,33] and CD.[17,21,26,28,30,32,37] Subgroup analysis of 3 trials comparing probiotics with 5-ASA[19,24,31] suggested that the effect of probiotics was comparable to 5-ASA in

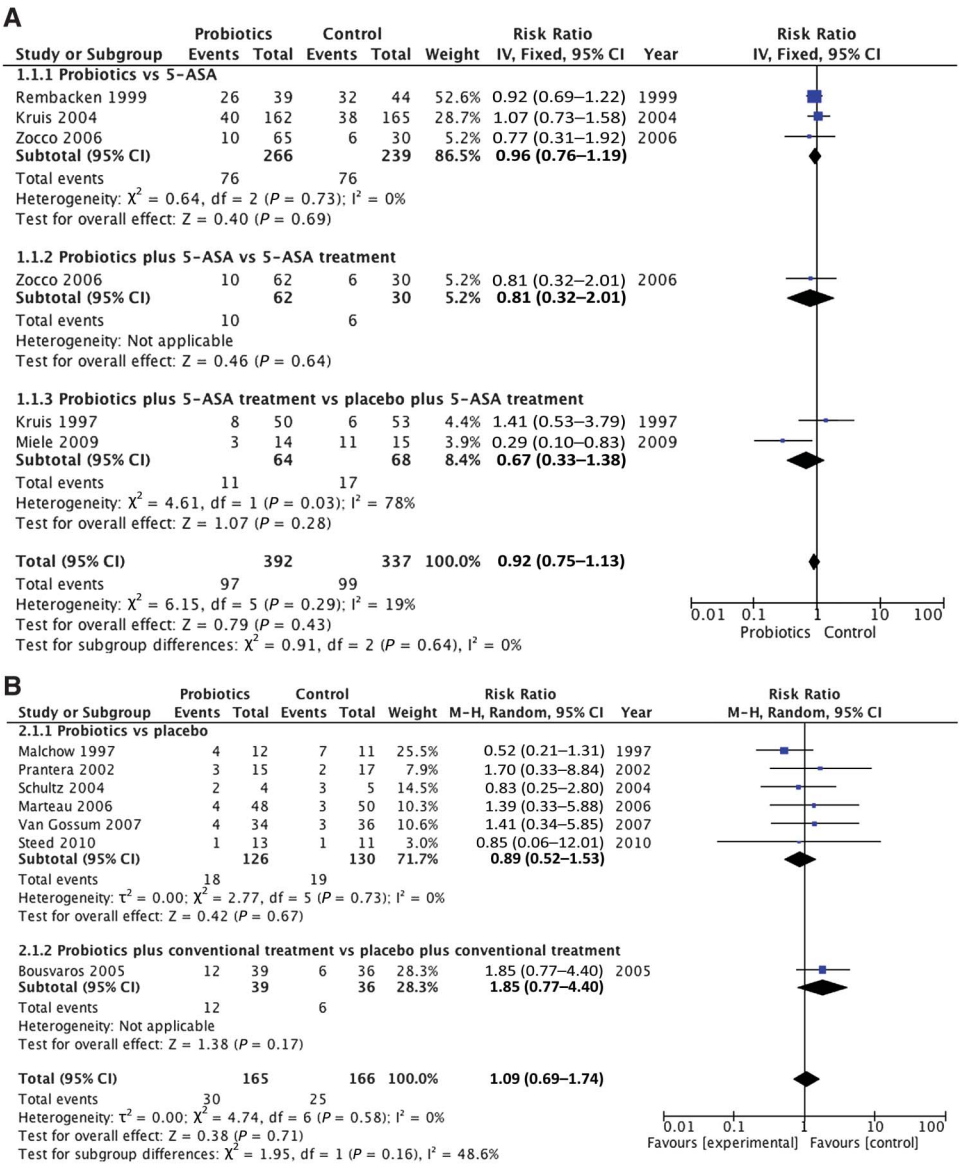

FIGURE 6. A, The subgroup analysis for the relapse rates of probiotics in maintaining remission of UC based on different trial design. B, The subgroup analysis for the relapse rates of probiotics in maintaining remission of CD based on different trial design.

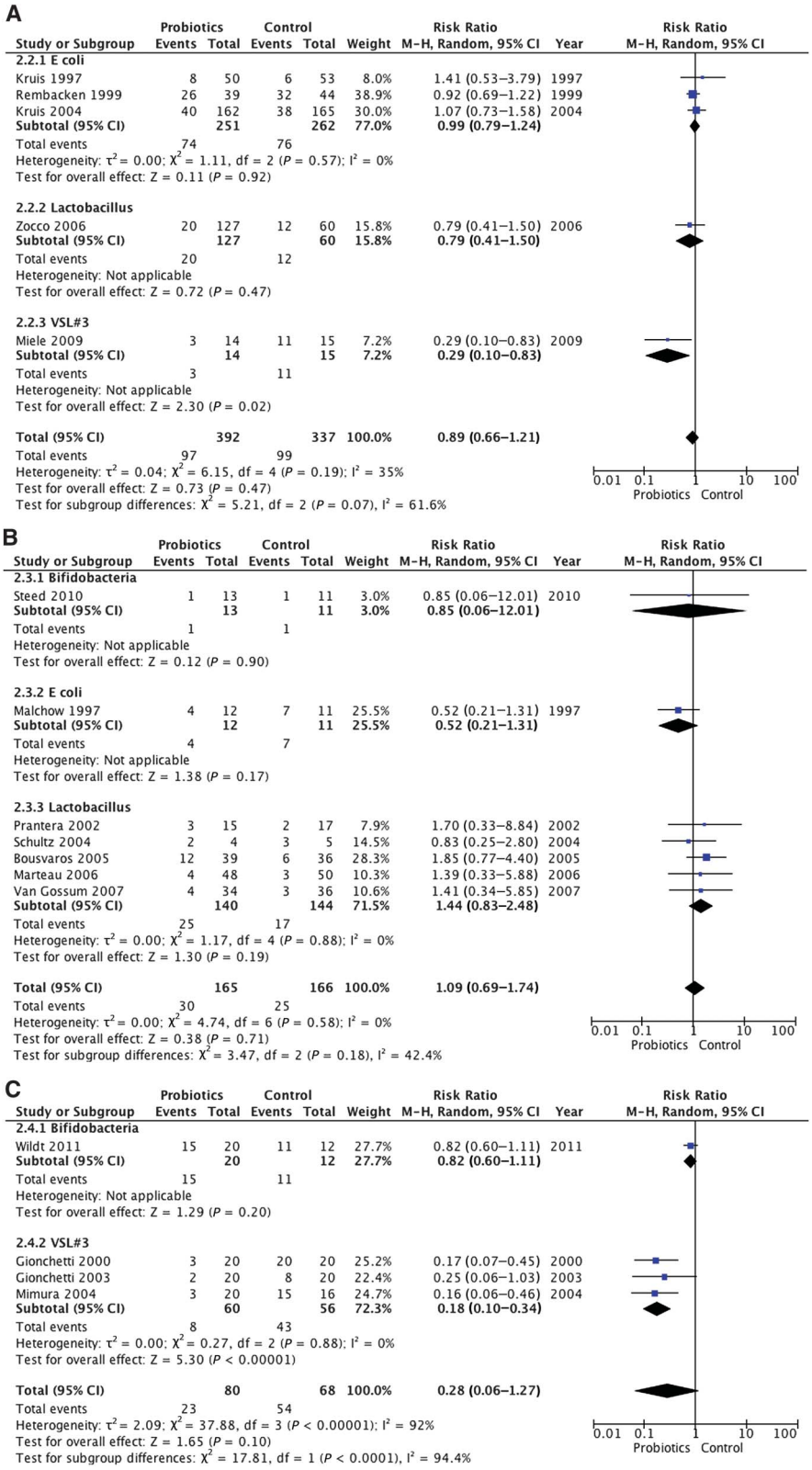

FIGURE 7. A, The subgroup analysis for the relapse rates of different probiotics in maintaining remission of UC. B, The subgroup analysis for the relapse rates of different probiotics in maintaining remission of CD. C, The subgroup analysis for the relapse rates of different probiotics in maintaining remission of pouchitis.

maintaining therapy in UC ($P = 0.69$, RR = 0.96), and the heterogeneity was not significant ($P = 0.73$, $I^2 = 0\%$) (Fig. 6A). Treatment of probiotics plus 5-ASA[18,33] did not have advantage over placebo plus 5-ASA for preventing relapse in UC ($P = 0.28$, RR = 0.67), although some heterogeneity was indicated ($P = 0.03$, $I^2 = 78\%$) (Fig. 6A). In maintaining therapy for CD, the results from 6 trials[17,21,26,30,32,37] suggested that administration of probiotics and placebo had no significant difference ($P = 0.67$, RR = 0.89), with little heterogeneity indicated ($P = 0.73$, $I^2 = 0\%$) (Fig. 6B).

Clinical Relapse in Maintaining Therapy in Inflammatory Bowel Disease with Different Probiotics

Because the varieties of probiotics used for maintaining therapy in IBD, we also carried out subgroup analysis based on different probiotics. The trial conducted by Miele et al[33] suggested that *VSL#3* had significant effect in maintaining therapy for patients with UC ($P = 0.02$, RR = 0.29) (Fig. 7A). Treatment with *E. coli*[31] or *Lactobacillus*[18,19,24] had comparable results as controls in trials on UC, with the *P* value 0.92 and 0.47, respectively (Fig. 7A). Although *Bifidobacteria*,[37] *E. coli,*[17] and *Lactobacillus*[21,26,28,30,32] were used for maintaining therapy in different trials, they did not show any favorable effect over controls in patients with CD (Fig. 7B). Meta-analyses of 3 trials[20,22,25] suggested that *VSL#3* significantly prevented clinical relapse in patients with pouchitis ($P < 0.00001$, RR = 0.20), and there was little heterogeneity ($P = 0.88$, $I^2 = 0\%$) (Fig. 7C).

Adverse Events

We combined 10 eligible trials and analyzed the adverse events of probiotics.[18,19,21,24,28,30,32,34,35,38] Meta-analysis of these 10 trials showed no significant difference between the interventions ($P = 0.69$, RR = 0.96) without significant heterogeneity ($P = 0.29$, $I^2 = 17\%$) (Fig. 8). Also, the difference between the interventions was not significant in UC ($P = 0.94$, RR = 0.99) or CD ($P = 0.33$, RR = 0.87) subgroup (Fig. 8).

DISCUSSION

The results from our meta-analyses of RCTs show a few implications for probiotics administration during consecutive processes in IBD treatment. Probiotics showed therapeutic benefit in inducing remission of UC in the present meta-analyses. We also found that maintaining remission of IBD with the probiotics reduced the recurrence and might be as effective as with 5-ASA, although the choice of probiotic bacteria, the optimal dose, mode of administration, and duration of therapy still need to be established. Recent evidence has suggested the potential therapeutic role for probiotics in the prevention or treatment of IBD. Several mechanisms have been elucidated, including restoring the microbial balance, modulating mucosal protection, protecting against pathogens,[40] inducing protective immune responses through immunization,[41] and modifying gut-associated lymphoid cells.[42] However, clinical results of probiotics for IBD remain controversial. There is still considerable work to do before probiotics can be considered as part of the standard treatment of IBD.

We found additional benefits of probiotics supplementation on inducing remission for UC. Significant difference was suggested between probiotics and placebo as supplemental treatment. Some results have demonstrated that probiotics could significantly prevent the initial injury of colitis.[43] Although a system review including limited articles has suggested promising results of probiotics, especially *VSL#3*, for inducing remission in active UC,[44] more experimental and clinical studies are needed to explain the

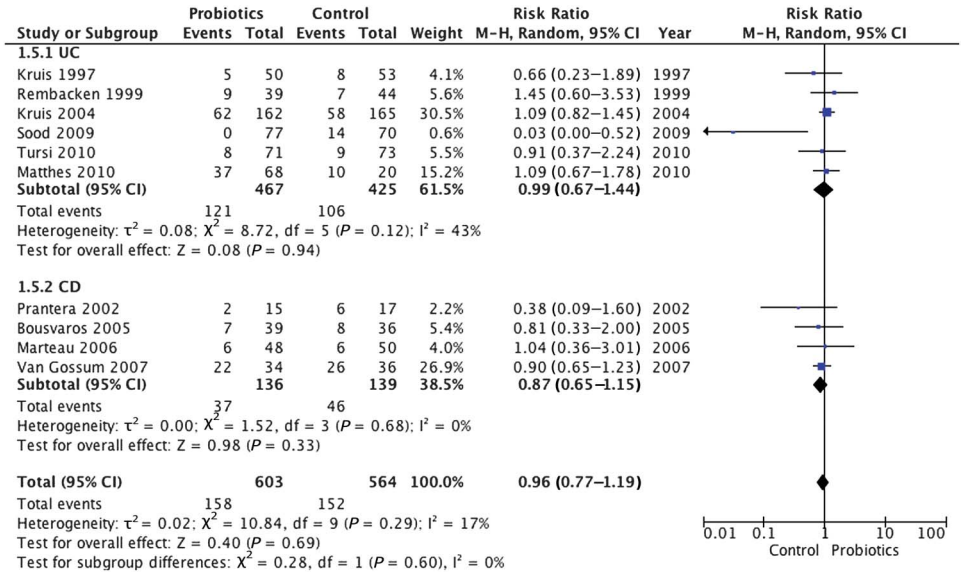

FIGURE 8. The forest plot of the adverse events because of probiotics or control groups in IBD.

different effects on probiotics between UC and CD. Interestingly, although the mechanisms are not fully understood, possible pathways have been preliminarily studied in models of UC. Administration of *VSL#3* results in a decrease of tumor necrosis factor-α, IL-6 and an increase of IL-10, which may due to exert the anti-inflammatory activity by inhibiting PI3K/Akt and NF-κB pathway.[45] Besides, *VSL#3* therapy protects the epithelial barrier and prevents the tight junction protein downregulation through activating the p38 and ERK signaling pathways.[46,47] The superior efficacy of *VSL#3* might be explained that the combined use of multiple probiotic strains (*VSL#3* contains 8 probiotic strains) has a stronger barrier-preserving effect than single probiotic strain alone.[47]

Maintenance therapy in IBD and prevention therapy, and the treatment of pouchitis, have emerged as areas in which probiotic therapy offers a valid therapeutic alternative to current treatments.[48] Our results indicated that maintaining remission of IBD with probiotics were as effective as with 5-ASA and superior to placebo. Furthermore, the need to combine 5-ASA with probiotics for maintaining treatment of IBD was insufficient. However, no additional benefit of probiotics administration on endoscopic relapse for CD was indicated. Fortunately, no more adverse events were suggested when compared with control groups in inducing remission or maintaining remission of IBD.

Probiotics have been investigated in clinical trials as treatments for IBD with conflicting results. Despite our results, 1 uncontrolled trial suggested that maintaining remission of UC with the probiotic *E. coli* Nissle 1917 was as effective as with standard mesalazine.[49] Nonpathogenic *E. coli* develops antagonistic activity against enterobacteria such as *Salmonella enteritidis, Shigella dysenteriae, Yersinia enterocolitica, and Vibrio cholerae*.[50] It can prevent the invasion of *Salmonella typhimurium* into intestinal cells, inhibit adhesion and invasion of adherent invasive *E. coli*,[51] and reduce concentrations of mucosa-associated colonic microflora constituents in UC.[52] In this study, clinical trials[18,19,24] have demonstrated similar effects in maintaining therapy for *E. coli* compared with 5-ASA. Probiotics are expected to apply to normalization of the intestinal flora, particularly the enhancement of *Bifidobacteria* in UC. However, the immunological modification by probiotic mixture in maintaining remission remains to be complicated. In vitro studies *Bifidobacteria* reduces mucosal inflammation and downregulates some proinflammatory cytokines.[53] These features could explain the efficacy of oral bacteriotherapy with *Bifidobacteria* as UC maintenance treatment.

The evidence for the use of probiotics in maintenance treatment of pouchitis is controversial. Trials of probiotics in CD were still less convincing as well. However, several studies have indicated their effectiveness in UC. There are several possible explanations for the different results on probiotics for UC and CD. First, Crohn's disease intestinal $CD4^+$ T cells display a proinflammatory cytokine profile with impaired production of the regulatory cytokine IL-10, whereas probiotics failed to restore this regulatory defect.[54] Second, patients with CD have circulating antibodies against bacterial flagellar proteins of enterobacteria and clostridia. In UC, there is less evidence for immune response to bacteria, but some changes including a relative deficiency of *Bifidobacteria* in gut microbiota are suggested.[55] Third, in contrast to UC, CD is characterized by an impaired induction of human beta defensins 2 and 3, which is deficient induction due to changes in the intracellular transcription by NF-kappaβ and the intracellular peptidoglycan receptor NOD2. These findings are consistent with the mucosal attachment of luminal bacteria in IBD and the frequent occurrence of other infectious agents.[56] However, we also found that the probiotic therapy *VSL#3* is highly effective in maintaining remission.[20,22,25,33] Tissue levels of tumor necrosis factor-α, interferon-γ, inducible nitric oxide synthase, and matrix metalloproteinases 2 and 9 can be reduced by *VSL#3*.[57] However, potential mechanisms for the beneficial effect of *VSL#3* for maintaining remission need to be further elucidated.

Limitations

There are several limitations to our analysis. First, despite our efforts to select trials with positive or negative results, we cannot rule out publication bias in our meta-analyses based on published studies. Second, only 3 trials[17,26,37] could be included to analyze the remission/response rate in CD, and sub-analyses was not applicable because of the heterogeneity of the studies. More rigorous and well-designed randomized controlled trials are needed to confirm our results. Finally, although we carefully selected outcome measures that were as conclusive and coherent as possible, the studies still had slightly different criteria for defining the outcomes of interests, which increased potential heterogeneity in our meta-analysis.

CONCLUSIONS

Probiotics showed therapeutic benefit in inducing remission of UC. It was also found that maintaining remission of UC with the probiotics might be as effective as with 5-ASA. *VSL#3* was also beneficial for maintaining remission in patients with pouchitis. However, no sufficient evidence suggested a significant benefit of probiotics for CD. *VSL#3* was superior to single strain both in inducing remission of UC and maintaining remission of pouchitis.

REFERENCES

1. Kayama H, Takeda K. Regulation of intestinal homeostasis by innate and adaptive immunity. *Int Immunol.* 2012;24:673–680.
2. Shanahan F. Physiological basis for novel drug therapies used to treat the inflammatory bowel diseases I. Pathophysiological basis and prospects for probiotic therapy in inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol.* 2005;288:G417–G421.
3. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology*. 2004;126:1620–1633.
4. Rosenstiel P. Stories of love and hate: innate immunity and host-microbe crosstalk in the intestine. *Curr Opin Gastroenterol.* 2013;29:125–132.
5. Dotan I, Rachmilewitz D. Probiotics in inflammatory bowel disease: possible mechanisms of action. *Curr Opin Gastroenterol.* 2005;21:426–430.
6. Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr.* 2007;137:819S–824S.
7. Macho Fernandez E, Valenti V, Rockel C, et al. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut*. 2011;60:1050–1059.
8. Peran L, Sierra S, Comalada M, et al. A comparative study of the preventative effects exerted by two probiotics, Lactobacillus reuteri and Lac-

tobacillus fermentum, in the trinitrobenzenesulfonic acid model of rat colitis. *Br J Nutr.* 2007;97:96–103.
9. Rutgeerts P, Vermeire S, Van Assche G. Mucosal healing in inflammatory bowel disease: impossible ideal or therapeutic target? *Gut*. 2007;56:453–455.
10. Seksik P, Sokol H, Lepage P, et al. Review article: the role of bacteria in onset and perpetuation of inflammatory bowel disease. *Aliment Pharmacol Ther.* 2006;24(suppl 3):11–18.
11. Ruseler-van Embden JG, Schouten WR, van Lieshout LM. Pouchitis: result of microbial imbalance? *Gut*. 1994;35:658–664.
12. Klinge LG, Kjeldsen J. Probiotics-should we change the treatment strategy for pouchitis? *Ugeskr Laeger*. 2006;168:3516–3518.
13. Gionchetti P, Rizzello F, Lammers KM, et al. Antibiotics and probiotics in treatment of inflammatory bowel disease. *World J Gastroenterol.* 2006; 12:3306–3313.
14. Naidoo K, Gordon M, Fagbemi AO, et al. Probiotics for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev.* 2011;(12): CD007443.
15. Butterworth AD, Thomas AG, Akobeng AK. Probiotics for induction of remission in Crohn's disease. *Cochrane Database Syst Rev.* 2008;(3): CD006634.
16. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials.* 1996;17:1–12.
17. Malchow HA. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol.* 1997;25:653–658.
18. Kruis W, Schütz E, Fric P, et al. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther.* 1997;11:853–858.
19. Rembacken BJ, Snelling AM, Hawkey PM, et al. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet*. 1999;354:635–639.
20. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology*. 2000;119:305–309.
21. Prantera C, Scribano ML, Falasco G, et al. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut*. 2002;51:405–409.
22. Gionchetti P, Rizzello F, Helwig U, et al. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology*. 2003;124:1202–1209.
23. Kato K, Mizuno S, Umesaki Y, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther.* 2004;20:1133–1141.
24. Kruis W, Fric P, Pokrotnieks J, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut*. 2004;53:1617–1623.
25. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut*. 2004;53:108–114.
26. Schultz M, Timmer A, Herfarth HH, et al. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol.* 2004;4:5.
27. Tursi A, Brandimarte G, Giorgetti GM, et al. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med Sci Monit.* 2004;10:PI126–PI131.
28. Bousvaros A, Guandalini S, Baldassano RN, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis.* 2005;11:833–839.
29. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*. 2005;54:242–249.
30. Marteau P, Lémann M, Seksik P, et al. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut*. 2006;55:842–847.
31. Zocco MA, dal Verme LZ, Cremonini F, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther.* 2006;23:1567–1574.
32. Van Gossum A, Dewit O, Louis E, et al. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis.* 2007;13:135–142.
33. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol.* 2009;104:437–443.
34. Sood A, Midha V, Makharia GK, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol.* 2009;7:1202–1209.
35. Matthes H, Krummenerl T, Giensch M, et al. Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered Escherichia coli Nissle 1917 (EcN). *BMC Complement Altern Med.* 2010;10:13.
36. Ng SC, Plamondon S, Kamm MA, et al. Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis. *Inflamm Bowel Dis.* 2010;16:1286–1298.
37. Steed H, Macfarlane GT, Blackett KL, et al. Clinical trial: the microbiological and immunological effects of synbiotic consumption - a randomized double-blind placebo-controlled study in active Crohn's disease. *Aliment Pharmacol Ther.* 2010;32:872–883.
38. Tursi A, Brandimarte G, Papa A, et al. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol.* 2010;105:2218–2227.
39. Wildt S, Nordgaard I, Hansen U, et al. A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12 for maintenance of remission in ulcerative colitis. *J Crohns Colitis.* 2011;5:115–121.
40. Dongarrà ML, Rizzello V, Muccio L, et al. Mucosal immunology and probiotics. *Curr Allergy Asthma Rep.* 2013;13:19–26.
41. Kawashima T, Hayashi K, Kosaka A, et al. Lactobacillus plantarum strain YU from fermented foods activates Th1 and protective immune responses. *Int Immunopharmacol.* 2011;11:2017–2024.
42. Fedorak RN, Madsen KL. Probiotics and the management of inflammatory bowel disease. *Inflamm Bowel Dis.* 2004;10:286–299.
43. Drouault-Holowacz S, Foligné B, Dennin V, et al. Anti-inflammatory potential of the probiotic dietary supplement Lactibiane Tolérance: in vitro and in vivo considerations. *Clin Nutr.* 2006;25:994–1003.
44. Jonkers D, Penders J, Masclee A, et al. Probiotics in the management of inflammatory bowel disease: a systematic review of intervention studies in adult patients. *Drugs*. 2012;72:803–823.
45. Dai C, Zheng CQ, Meng FJ, et al. VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. *Mol Cell Biochem.* 2013;374:1–11.
46. Mennigen R, Nolte K, Rijcken E, et al. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G1140–G1149.
47. Dai C, Zhao DH, Jiang M. VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. *Int J Mol Med.* 2012;29:202–208.
48. Fedorak R, Demeria D. Probiotic bacteria in the prevention and the treatment of inflammatory bowel disease. *Gastroenterol Clin North Am.* 2012; 41:821–842.
49. Adam B, Liebregts T, Holtmann G. Maintaining remission of ulcerative colitis with the probiotic Escherichia Coli Nissle 1917 is as effective as with standard mesalazine. *Z Gastroenterol.* 2006;44:267–269.
50. Schulze J, Sonnenborn U. Oral administration of a certain strain of live Escherichia coli for intestinal disorders? *Infection*. 1995;23:184–186.
51. Boudeau J, Rich C, France CF, et al. Escherichia coli strain Nissle 1917 inhibits adhesion to and invasion of intestinal epithelial cells by adherent-invasive E. coli isolated from a Crohn's disease patient. *Gastroenterology*. 2001;120(suppl):A190.
52. Swidsinski A, Swidsinski S, Godzun A, et al. Therapy with E. coli Nissle reduces concentrations of mucosa associated colonic flora in patients with ulcerative colitis. *Gastroenterology*. 2000;118(suppl):A1138.
53. Nanda Kumar NS, Balamurugan R, Jayakanthan K, et al. Probiotic administration alters the gut flora and attenuates colitis in mice administered dextran sodium sulfate. *J Gastroenterol Hepatol.* 2008;23:1834–1839.

54. Hvas CL, Kelsen J, Agnholt J, et al. Crohn's disease intestinal CD4+ T cells have impaired interleukin-10 production which is not restored by probiotic bacteria. *Scand J Gastroenterol.* 2007;42: 592–601.
55. Subramanian S, Campbell BJ, Rhodes JM. Bacteria in the pathogenesis of inflammatory bowel disease. *Curr Opin Infect Dis.* 2006;19: 475–484.
56. Fellermann K, Wehkamp J, Herrlinger KR, et al. Crohn's disease: a defensin deficiency syndrome? *Eur J Gastroenterol Hepatol.* 2003;15: 627–634.
57. Ulisse S, Gionchetti P, D'Alò S, et al. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am J Gastroenterol.* 2001; 96:2691–2699.