Dig Dis Sci (2008) 53:2524–2531
DOI 10.1007/s10620-007-0171-0

ORIGINAL PAPER

A Meta-Analysis on the Efficacy of Probiotics for Maintenance of Remission and Prevention of Clinical and Endoscopic Relapse in Crohn's Disease

Roja Rahimi · Shekoufeh Nikfar · Fatemeh Rahimi · Behzad Elahi · Saeed Derakhshani · Mohammad Vafaie · Mohammad Abdollahi

Received: 4 July 2007 / Accepted: 20 December 2007 / Published online: 14 February 2008

Abstract *Objective* To evaluate whether probiotics maintain remission in patients with Crohn's disease (CD). *Design* A meta-analysis of controlled clinical trials. *Methods* PUBMED and Cochrane Central Register of Controlled Trials were searched for clinical trial studies investigated the efficacy of probiotics for the maintenance of remission in Crohn's disease. Clinical relapse and endoscopic relapse were the key outcomes of interest. Data were searched within the time period of 1966 through may 2007. *Result* Eight randomized placebo-controlled clinical trials met our criteria and were included in the analysis. Seven determined clinical relapse and three evaluated endoscopic relapse among patients with CD received probiotics for maintenance of remission. Pooling of seven trials for the outcome of clinical relapse yielded an odds ratio of 0.92 (95% confidence interval of 0.52–1.62, $P = 0.8853$), a nonsignificant odds ratio. The odds ratio for three studies for the outcome of endoscopic relapse was 0.97 (95% confidence interval of 0.54–1.78, $P = 0.93$), a nonsignificant odds ratio. *Conclusion* This meta-analysis fails to demonstrate the efficacy of probiotics in maintaining remission and preventing clinical and endoscopic recurrence in CD. It is suggested to use probiotic preparations containing a mixture of lactobacillus with *E. coli* or *Saccharomyces*.

Keywords Crohn's disease · Probiotics · Clinical relapse · Endoscopic relapse · Meta-analysis

R. Rahimi · F. Rahimi
Pharmaceutical Sciences Research Center,
Tehran University of Medical Sciences, Tehran, Iran

S. Nikfar
Drug Selecting Committee, Food and Drug Organization, and
Food and Drug Laboratory Research Center,
Ministry of Health and Medical Education, Tehran, Iran

B. Elahi
School of Medicine, Tehran University of Medical Sciences,
Tehran, Iran

S. Derakhshani · M. Vafaie
Baharan Coloproctology Surgical Clinic, Tehran, Iran

M. Abdollahi (✉)
Faculty of Pharmacy, and Pharmaceutical Sciences Research
Center, Tehran University of Medical Sciences (TUMS),
P.O. Box 14155-6451, Tehran, Iran
e-mail: mohammad.abdollahi@utoronto.ca

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease characterized by discontinuous transmural inflammation affecting any portion of the gastrointestinal tract, from the mouth to the anus [1].

The natural history of CD is characterized by recurrent flare-ups of symptoms and by postoperative recurrence after curative surgery [2]. More than 70% of CD patients are operated at least once during their lifetime. New lesions recur early after ileocolonic resection and this often leads to clinical recurrence and eventual reoperation. Median time to surgical recurrence is 10–20 years, to clinical recurrence 3–5 years, and to endoscopic recurrence 6 months [3].

Prevention of relapse is a key objective in the management of CD. There is no current treatment available that completely maintains remission without significant side-effects. 5-Aminosalicylate (5-ASA) preparations are frequently used to maintain remission but there is no evidence to suggest that 5-ASA preparations are superior to placebo in maintaining medically induced remission [4]. Corticosteroids are also evaluated for maintenance remission in CD and do not appear to reduce the risk of relapse [5].

Recently, probiotics have been concerned as a treatment for maintaining remission and preventing recurrence of CD. Probiotics are living microorganisms that, upon ingestion in sufficient numbers, exert benefits on human health [6]. The bacteria associated with probiotic activity have frequently been lactobacilli or bifidobacteria, but *Escherichia coli* and *enterococcal* strains have been used, as have nonbacterial organisms such as *Saccharomyces boulardii*. The rationale for using probiotics in the treatment of CD is based on convincing evidence implicating intestinal bacteria in the pathogenesis of the disease. It appears that the pathogenesis of CD involves genetically influenced dysregulation of the mucosal immune response to antigens present in the normal bacterial flora [7]. The patient's endogenous bacterial flora may initiate a cascade, resulting in intestinal injury by secreting inflammatory mediators such as lipopolysaccharide, which may activate the host's innate immune system and initiate the aberrant immune response [8]. Mechanisms by which probiotics exert their therapeutic effects include: (1) modulation of barrier function, (2) antagonistic activity against pathogenic bacteria either by inhibition of adherence and translocation or by production of antibacterial substances, (3) modulation of intestinal cytokine production, (4) anti-inflammatory properties, and (5) improvement of gut permeability [6, 9].

The results obtained from studies evaluating the efficacy of probiotics in maintaining remission in CD are conflicting [10, 11]. Although narrative reviews have been used for this purpose, these are largely subjective and thus different experts can come to different conclusions and it becomes impossibly difficult when more than a few studies are involved. On the other hand, decision making on the basis of clinical trials with different results is too hard and possibly mistaken, which is why the performance of a meta-analysis is necessary. Meta-analysis is a statistical procedure for combining data from multiple studies to reach a better conclusion because only summary statistics are available in the literature. Only one systematic review has been performed in this field, published in 2006, with the inclusion of seven trials from the period 1966–2005 [12]; it reached the conclusion that probiotics are ineffective in the improvement of CD. In the present meta-analysis we collected all studies on the effects of probiotics on CD in the period from 1966 to May 2007 to update information about the effectiveness of probiotics.

Methods

Data Sources and Study Selection

PubMed and Cochrane Central Register of Controlled Trials were searched for studies investigated the efficacy of probiotics in maintaining remission in patients with CD. Data were collected from 1966 to May 2007. The search terms were: "probiotic", "probiotics", "*Escherichia coli*", "lactobacillus", "bifidobacteria", "yeast", "Crohn", and "inflammatory bowel disease". There was no language restriction. The reference list from retrieved articles was also reviewed for additional applicable studies. Clinical relapse and endoscopic relapse were the key outcomes of interest. Randomized, placebo-controlled trials investigating the efficacy of probiotics in maintaining remission of CD were considered. Each article was reviewed to eliminate duplicates, reviews, case studies, and uncontrolled trials. Trials were disqualified if their outcome was not relapse (clinical and/or endoscopic), and those whose target groups were not patients with CD (patients with ulcerative colitis or pouchitis) were excluded from meta-analysis. We considered completed published studies as well as abstracts presented at meetings.

Data Extraction and Statistical Analysis

Data from selected studies were extracted in the form of 2×2 tables. All included studies were pooled and weighted. The data were analyzed using Statsdirect (2.6.1). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using the Mantel-Haenszel method. The Breslow-Day test was used to test for heterogeneity. The event rate in the experimental (intervention) group against the event rate in the control group was calculated using a L'Abbe plot as an aid to explore the heterogeneity of effect estimates. Funnel plot analysis was used as a publication bias indicator.

Results

The electronic searches yielded 2307 items; 2270 from PubMed, and 37 from Cochrane Central. Of those, 11 trials were scrutinized in full text. Three reports were considered ineligible. Thus, eight trials were included in the analysis [10, 11, 13–17] (Fig. 1). These included 320 patients with CD randomized to receive either probiotics or placebo. Patient characteristics, type and dosage of probiotic, duration of treatment, interventions, and method of study are presented in Table 1. Outcomes of clinical relapse and endoscopic relapse were determined in seven [10, 11, 13, 14, 16–18] and three [11, 15, 16] trials, respectively.

As shown in Tables 2 and 3, clinical relapse in the probiotic group was 24.6% (32/130) and in the placebo group was 26.8% (33/123); endoscopic relapse in the probiotic group was 58% (50/86) and in the placebo group was 58% (53/91).

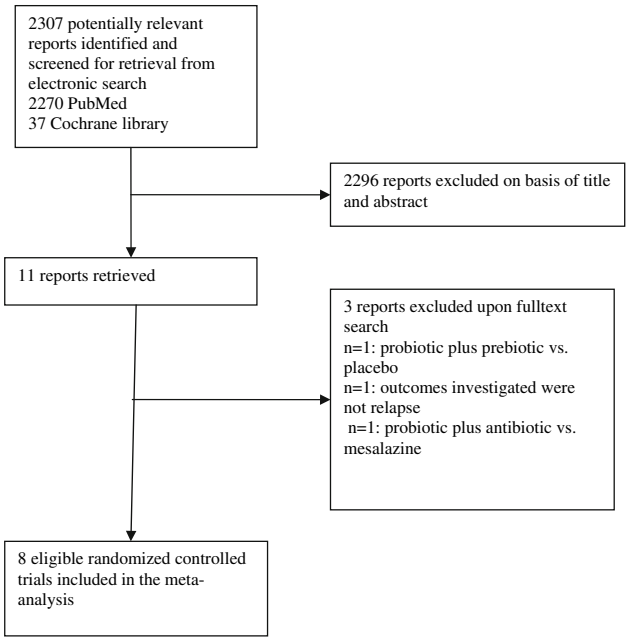

Fig. 1 Flow diagram of the trial selection process

The summary OR for relapse of disease outcomes by clinical evaluation among probiotics intake in seven trials [10, 11, 13, 14, 16–18] was 0.920454 with a 95% CI of 0.523229–1.619244, indicating a nonsignificant OR ($P = 0.8853$, Fig. 2). The Breslow-Day test for heterogeneity ($P = 0.098$) indicated that the studies were not heterogeneous and could be combined, thus the fixed effects for individual and summary of OR for meta-analysis of studies have been applied (Fig. 3).

Regression of normalized effect versus precision for all included studies for "relapse with probiotics therapy" was −3.297887 (95% CI = −6.069032 to −0.526741, $P = 0.0281$), and Kendall's test on standardized effect versus variance indicated tau = −0.619048, $P = 0.0302$ (Fig. 4).

The summary OR for relapse of disease outcomes by endoscopic evaluation among probiotics intake in three trials [11, 15, 16] was 0.978567 with a 95% CI of 0.539186–1.775999, indicating a nonsignificant OR ($P = 0.9353$, Fig. 5). The Breslow-Day test for heterogeneity ($P = 0.108$) indicated that the studies were not heterogeneous and could be combined, thus the fixed effects for individual and summary of OR for meta-analysis of studies have been applied (Fig. 6).

For four studies [11, 13, 14, 17] for which data for relapse of disease outcomes among probiotics intake (*Lactobacillus rhamnosus* strain GG = LGG) therapy could

Table 1 Characteristics of studies evaluating the efficacy of probiotics in preventing recurrence of CD

Trial	Age (year)		Sex (M/F)		Probiotics	Probiotic dosage	Duration (month)	Interventions	Method
	Probiotic	Placebo	Probiotic	Placebo					
Bousvaros et al. [13]	14.8	14.9	26/13	21/15	LGG	2×10^{10} (cfu/day)	24	Aminosalicylates, 6-mercaptopurine, azathioprine Low-dose alternate-day corticosteroids (<0.5 mg/kg every other day)	Multicenter (11 center), double blind
Malchow [18]	–	–	–	–	*E. coli*	2.5×10^{10} (cfu/day)	12	Prednisolone (60 mg/day) tapering to 5 mg/day	Single center, double blind
Schultz et al. [14]	–	–	–	–	LGG	2×10^{9} (cfu/day)	6	Ciprofloxacin 500 mg bid, metronidazole 250 mg tid	Single center, double blind
Prantera et al. [11]	37.3	36.2	14/9	15/7	LGG	12×10^{9} (cfu/day)	13	–	Single center, double blind
Guslandi et al. [10]	39.5	35.5	9/7	11/5	SB	1 g/day	6	Mesalamine 1g tid or bid	Single center, investigator blind
Marteau et al. [15]	32	29	26/22	21/29	LA_1	4×10^{9} (cfu/day)	6	Corticosteroids	Multicenter (16 center), double blind
Van Gossum et al. [16]	38.7	35	19/15	18/18	LA_1	10^{10} (cfu/day)	3	Amoxiciline/clavulanic 500 mg tid	Multicenter (6 center), double blind
Zocco et al. [17]	–	–	–	–	LGG	18×10^{9} (cfu/day)	12	Mesalazine 2.4 g/day	Single center, unblinded

Lactobacillus johnsonii (LA_1); *Lactobacillus rhamnosus* strain GG (LGG); *Escherichia coli* strain nissle 1917 (*E. coli*); *Saccharomyces boulardii* (SB)

Table 2 Outcome of clinical relapse

Study	Definition of clinical recurrence	Clinical relapse (based on CDAI score)	
		Probiotic	Placebo
Bousvaros et al. [13]	PCDAI score of greater than 30 points on any single visit or a PCDAI score greater than 15 points of any two consecutive visit more than 1 week apart; or need for corticosteroid or other rescue therapy for active CD; or need for surgery or hospitalization for a complication of CD	12/39	6/36
Malchow et al. [18]	CDAI >150	3/10	7/10
Schultz et al. [14]	An increase in CDAI of >100 points	2/4	3/5
Prantera et al. [11]	An increase in CDAI to more than 150 points, confirmed by endoscopic signs of inflammation	8/23	5/22
Guslandi et al. [10]	CDAI >150 with an increase of 100 points over the baseline values for more than 2 weeks	1/16	6/16
Van Gossum et al. [16]	CDAI >150 with an increase of 70 points or higher from baseline	4/27	3/22
Zocco et al. [17]	CDAI score	2/11	3/12

Pediatric Crohn's Disease Activity Index (PCDAI); Crohn's Disease Activity Index (CDAI)

Table 3 Outcome of endoscopic relapse

Study	Definition of endoscopic recurrence	Endoscopic relapse (based on endoscopic findings)	
		Probiotic	Placebo
Prantera et al. [11]	Determined according to the Rutgeerts scoring system (presence of grade 2 or higher)	9/15	6/17
Marteau et al. [15]	Ileum lesions determined according to the Rutgeerts scoring system (grade >1) and colonic lesions by adapted classification	21/43	30/47
Van Gossum et al. [16]	Determined according to the Rutgeerts scoring system	20/28	17/27

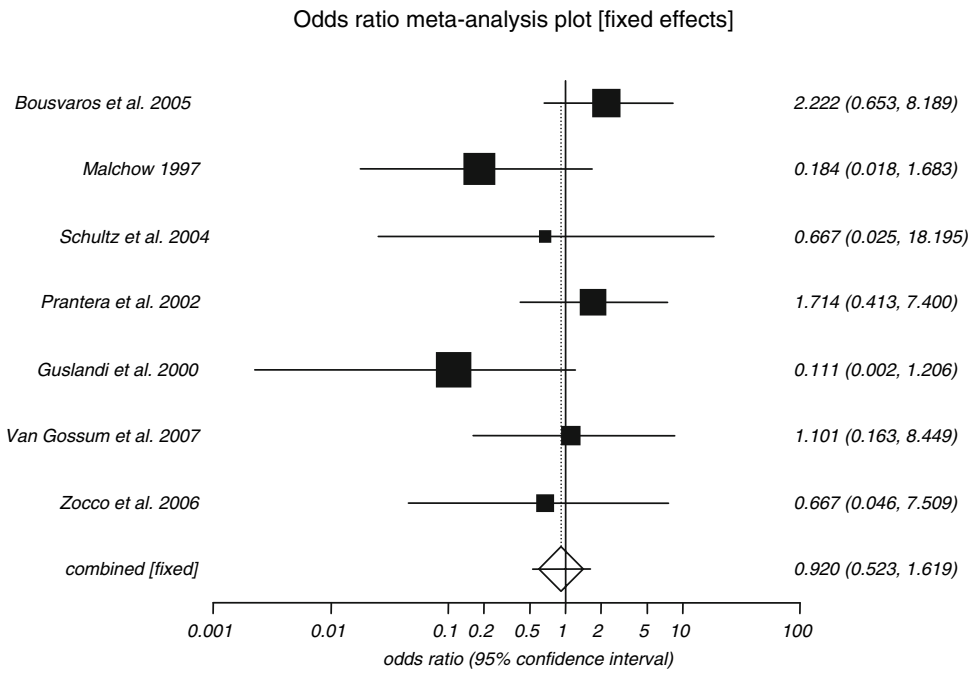

Fig. 2 Individual and pooled odds ratios for the outcome of "clinical relapse" in the studies considering probiotics therapy

be extracted, the summery OR was 1.572638 with a 95% CI of 0.761166–3.249213, a nonsignificant OR ($P = 0.2068$, Fig. 7).

The Breslow-Day test for heterogeneity ($P = 0.6775$) indicated that the studies were not heterogeneous and could be combined and the fixed effects for individual and

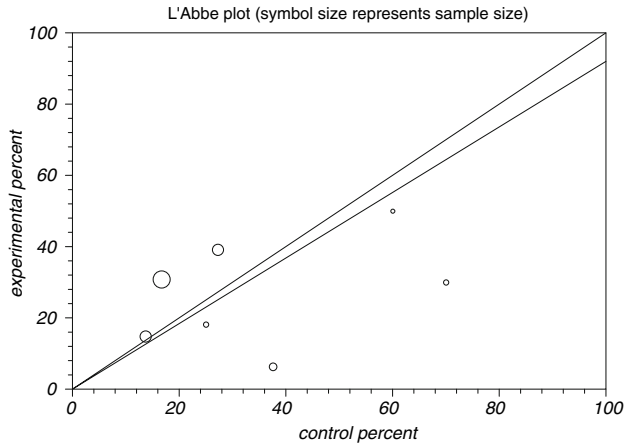

Fig. 3 Heterogeneity indicators for the outcome of "clinical relapse" for studies including probiotics therapy

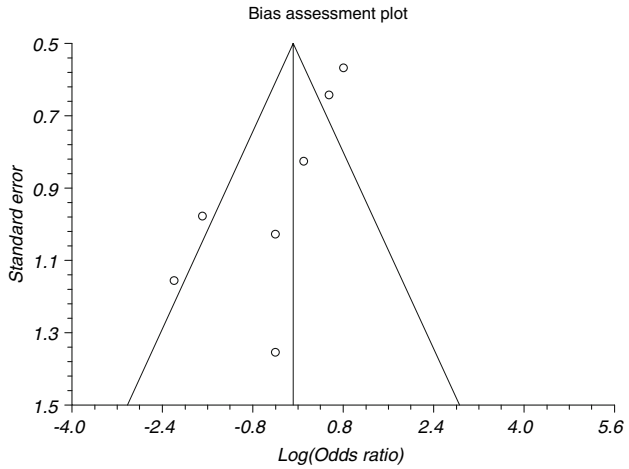

Fig. 4 Publication bias indicators for the outcome of "clinical relapse" for studies including probiotics therapy

summary of OR for meta-analysis of studies have been applied (Fig. 8).

Discussion

The natural history of CD includes periods of disease flare-up and remission, and treatment in CD is directed towards inducing and maintaining remission of symptoms. Although several drug regimens show efficacy in inducing clinical remission in active CD [19–21], no treatments are available to avoid relapse of the disease. Regarding the role of intestinal flora in pathophysiology of CD, it seems that probiotics may have potential to maintain remission in CD.

The results from this meta-analysis showed that probiotics are not more beneficial than placebo for maintaining remission and preventing clinical and endoscopic relapses in patients with CD. Analyzing the studies in which the probiotic preparation consisted of LGG suggests that LGG does not reduce the risk of clinical relapse.

Of the eight trials that were included for this meta-analysis, six trials [11, 13–17] claimed that probiotics are not superior to placebo in maintaining remission and could not prevent clinical and endoscopic relapses and only two [10, 18] suggested that probiotics could maintain remission and prevent clinical relapse. The present results are supported by the conclusion of a systematic review of the efficacy of probiotics in maintaining remission in CD

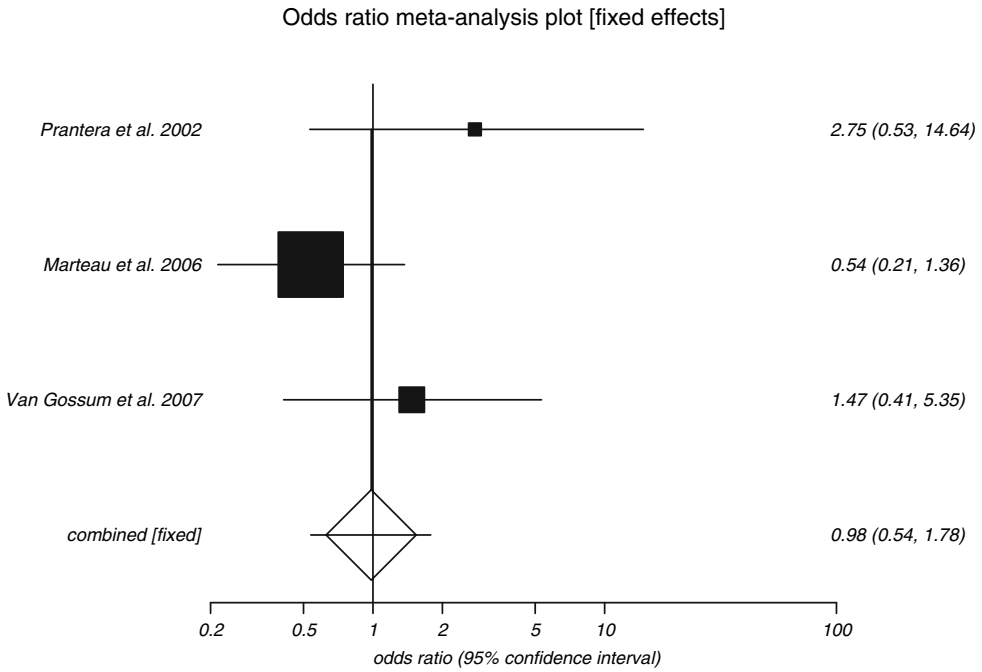

Fig. 5 Individual and pooled odds ratios for the outcome of "endoscopic relapse" in the studies considering probiotics therapy

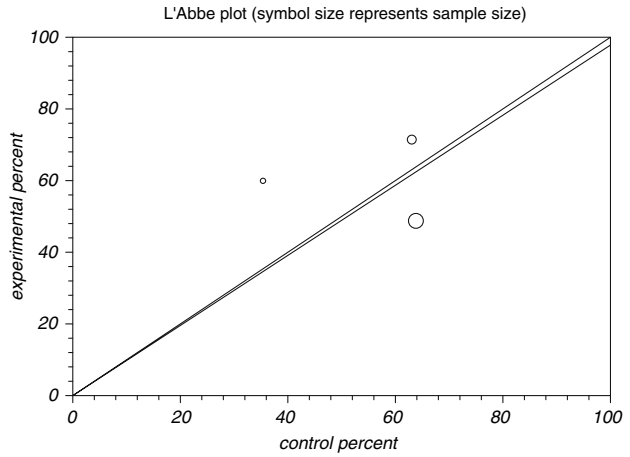

Fig. 6 Heterogeneity indicators for the outcome of "endoscopic relapse" for studies including probiotics therapy

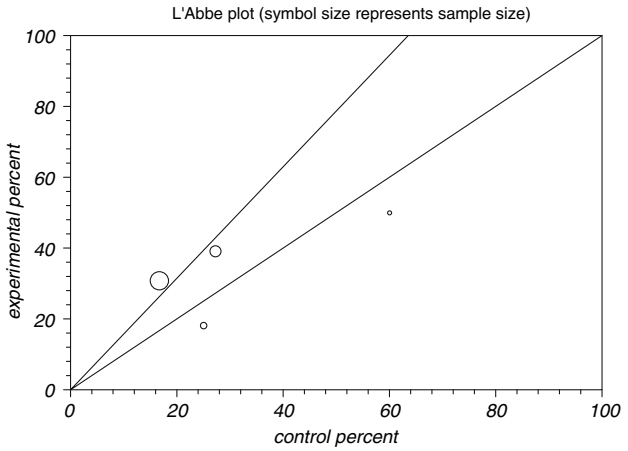

Fig. 8 Heterogeneity indicators for the outcome of "clinical relapse" for studies including probiotics therapy (*Lactobacillus rhamnosus* strain GG = LGG)

by Rolfe et al. [12], although some differences exist between two studies. In the previous meta-analysis, seven papers were included while we included eight papers. In addition, one trial that was included in the previous meta-analysis [22] was not considered eligible in our meta-analysis, because it compared rifaximine plus probiotic against mesalazine. We included two new studies instead [15, 16], which both determined the outcome of endoscopic relapse. The trial done by Van Gossum et al. also evaluated the outcome of clinical relapse [16]. The trial done by Bousvaros et al. [13] was analyzed separately in the previous analysis because it investigated the clinical relapse among pediatrics but we did not separate it from other studies. The trial of Guslandi et al. [10] was also analyzed individually because the probiotic preparation used in that study contained yeast other than bacteria. Furthermore, we improved the present work by reanalyzing the studies in which the probiotic preparation consisted of LGG.

It should not be forgotten that the type of probiotics differs and thus extrapolation of a positive or negative result obtained with one type to another type is challenging. Interestingly, in all trials claiming ineffectiveness of probiotics in preventing relapse, lactobacillus was the main composition [11, 13–17]. In addition, in two trials [10, 18]

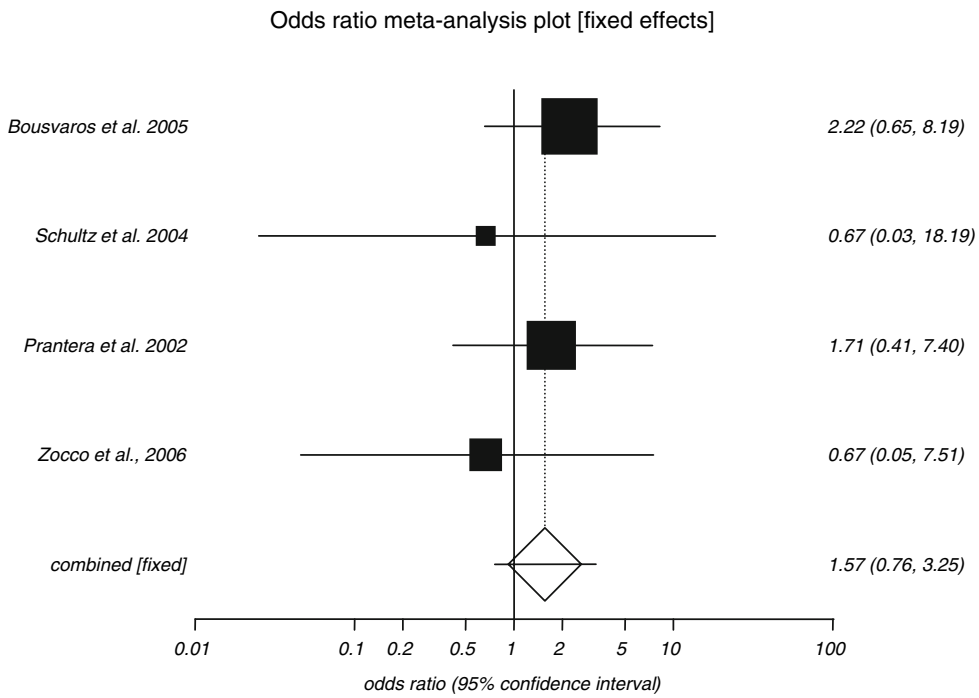

Fig. 7 Individual and pooled odds ratios for the outcome of "clinical relapse" in the studies considering probiotics therapy (*Lactobacillus rhamnosus* strain GG = LGG)

that concluded effectiveness of probiotics, the lactobacillus was not present. In one of them, *E. coli* and in the other one the yeast *Saccharomyces* were used in the probiotic preparation. Therefore it is obvious that probiotics containing lactobacillus alone cannot reduce the risk of relapse in patients with CD.

On the other hand, there is no dose-response study available for probiotics and the dose chosen in clinical trials is usually based on the amount present in dairy products. Thus, it is possible that the ineffectiveness of lactobacillus containing probiotics is due to an inappropriate dose of lactobacillus present in these preparations.

The effects of probiotics on the immune system are not fully understood. Malin et al. have suggested that LGG has the potential to increase the gut IgA immune response and thereby promote the gut immunological barrier [23]. Beyond this result, any form of bacterium can become an antigenic stimulus and consequently be the cause of the increased recurrences. In two studies [11, 13], it was seen that the recurrence rate tended to be higher in the probiotic group than in the placebo group.

One should also consider that the efficacy of one probiotic may not be the same in all patients or in the same patient at different stages of disease. Responsiveness to treatment is dependent on several variables, including the characteristics of the host (age, sex, lifestyle, compliance), the lesions (site, extent, type of gross lesions), previous history (presence, number and type of resections), and risk factors (smoking, appendectomy, familial history of inflammatory bowel disease) [2].

One might think that severity of disease or other complications in this kind of patients could confound the results of this study. In addition, some might think that the small number of included studies may reduce the impact of the meta-analysis. In answer to both of these questions, it should be emphasized that the most important parameter in meta-analysis is inclusion and it is necessary to select shared outcomes of different studies and exclude those that had different study design. Thus, the small number of included studies in the present meta-analysis does not bias its conclusion. All included studies were pooled and weighted, the OR was derived from outcomes, and a fixed effect model was used. In a fixed model, the true magnitude of the effect is assumed to be a constant whose value is unknown but is estimated by using the values from the included studies. Therefore, severity of disease or other confounding parameters cannot bias this meta-analysis.

In conclusion, this meta-analysis fails to demonstrate the efficacy of probiotics in maintaining remission and preventing clinical and endoscopic recurrence in CD. It is suggested to use probiotic preparations containing a mixture of lactobacillus with *E. coli* or *Saccharomyces*.

References

1. Fow J, Grossman S (2007) A comprehensive guide to patient-focused management strategies for Crohn disease. Gastroenterol Nurs 30:93–98
2. Biancone L, Tosti C, Fina D, Fantini M, De Nigris F, Geremia A, Pallone F (2003) Review article: maintenance treatment of Crohn's disease. Aliment Pharmacol Ther 17:S31–S37
3. Lemann M (2006) Review article: can post-operative recurrence in Crohn's disease be prevented? Aliment Pharmacol Ther 24: S22–S28
4. Akobeng AK, Gardener E (2005) Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's Disease. Cochrane Database Syst Rev 25:CD003715
5. Steinhart AH, Ewe K, Griffiths AM, Modigliani R, Thomsen OO (2003) Corticosteroids for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 4:CD000301
6. Jones JL, Foxx-Orenstein AE (2007) The role of probiotics in inflammatory bowel disease. Dig Dis Sci 52:607–611
7. Shanahan F (2003) Probiotics in inflamatory bowel disease. Gut 48:609
8. Sartor RB (2003) Targeting enteric bacteria in treatment of inflammatory bowel diseases: why, how, and when. Curr Opin Gastroenterol 19:358–365
9. Gionchetti P, Rizzello F, Campieri M (2002) Probiotics in gastroenterology. Curr Opin Gastroenterol 18:235–239
10. Guslandi M, Mezzi G, Sorghi M, Testoni PA (2000) Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig Dis Sci 45:1462–1464
11. Prantera C, Scribano ML, Falasco G, Andreoli A, Luzi C (2002) Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. Gut 51:405–409
12. Rolfe VE, Fortun PJ, Hawkey CJ, Bath-Hextall F (2006) Probiotics for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 18:CD004826
13. Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL (2005) 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 11:833–839
14. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC (2004) Lactobacillus GG in inducing and maintaining remission of Crohn's disease BMC Gastroenterol 4:5
15. Marteau P, Lemann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soule JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY (2006) Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. Gut 55: 842–847
16. Van Gossum A, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, DeVos M, Enslen M, Paintin M, Franchimont D (2007) 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 13:135–142
17. Zocco MA, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A (2006) Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 23:1567–1574

18. Malchow HA (1997) Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? J Clin Gastroenterol 25:653–658
19. Rahimi R, Nikfar S, Rezaie A, Abdollahi M (2006) A meta-analysis of broad-spectrum antibiotic therapy in patients with active Crohn's disease. Clin Ther 28:1983–1988
20. MacDonald JK, McDonald JW (2007) Natalizumab for induction of remission in Crohn's disease. Cochrane Database Syst Rev 1:CD006097
21. Kane SV, Schoenfeld P, Sandborn WJ, Tremaine W, Hofer T, Feagan BG (2002) The effectiveness of budesonide therapy for Crohn's disease. Aliment Pharmacol Ther 16:1509–1517
22. Campieri M, Rizzello F, Venturi A, Poggioli G, Ugolini F, Helwig U, Amadini C, Romboli E, Gionchetti P (2000) Combination of antibiotic and probiotic treatment in efficacious in prophylaxis of post-operative recurrence of crohn's disease: a randomized controlled study versus mezalamine. Gastroenterology 118:A781
23. Malin M, Suomalainen H, Saxelin M, Isolauri E (1996) Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG. Ann Nutr Metab 40:137–145