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The Effects of Probiotics and Symbiotics on Risk Factors for Hepatic Encephalopathy

A Systematic Review

Daniela Viramontes Hörner, MRes, Amanda Avery, BSc, and Ruth Stow, MRes

Abstract: Alterations in the levels of intestinal microbiota, endotoxemia, and inflammation are novel areas of interest in the pathogenesis of hepatic encephalopathy (HE). Probiotics and symbiotics are a promising treatment option for HE due to possible beneficial effects in modulating gut microflora and might be better tolerated and more cost-effective than the traditional treatment with lactulose, rifaximin or L-ornithine-L-aspartate. A systematic search of the electronic databases PubMed, ISI Web of Science, EMBASE, and Cochrane Library was conducted for randomized controlled clinical trials in adult patients with cirrhosis, evaluating the effect of probiotics and symbiotics in changes on intestinal microflora, reduction of endotoxemia, inflammation, and ammonia, reversal of minimal hepatic encephalopathy (MHE), prevention of overt hepatic encephalopathy (OHE), and improvement of quality of life. Nineteen trials met the inclusion criteria. Probiotics and symbiotics increased beneficial microflora and decreased pathogenic bacteria and endotoxemia compared with placebo/no treatment, but no effect was observed on inflammation. Probiotics significantly reversed MHE [risk ratio, 1.53; 95% confidence interval (CI): 1.14, 2.05; $P = 0.005$] and reduced OHE development (risk ratio, 0.62; 95% CI: 0.48, 0.80; $P = 0.0002$) compared with placebo/no treatment. Symbiotics significantly decreased ammonia levels compared with placebo (15.24; 95% CI: −26.01, −4.47; $P = 0.006$). Probiotics did not show any additional benefit on reversal of MHE and prevention of OHE development when compared with lactulose, rifaximin, and L-ornithine-L-aspartate. Only 5 trials considered tolerance with minimal side effects reported. Although further research is warranted, probiotics and symbiotics should be considered as an alternative therapy for the treatment and management of HE given the results reported in this systematic review.

Key Words: probiotics, symbiotics, hepatic encephalopathy, hepatic cirrhosis

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Hepatic cirrhosis is a global public health problem, with a continuing increase in its prevalence, incidence, hospitalizations, and mortality rate.[1–3] In addition, cirrhosis presents an economic burden with estimated cost to the United States health care system ranging from $14 million to $2 billion per year.[4] Hepatic encephalopathy (HE) is a serious and progressive neuropsychiatric abnormality in cirrhotic patients that significantly affects their quality of life and daily functioning. HE is split into overt hepatic encephalopathy (OHE), the severe form ranked in 4 different grades according to the West Haven criteria,[5] and minimal HE (MHE), the earliest or subclinical form, which can be a marker of the development of OHE.[6–8] The pathogenesis of HE is only partly understood. It has been suggested that ammonia production plays a main role; nevertheless, alterations in the levels of gut microflora [eg, small intestinal bacterial overgrowth (SIBO)], which lead to endotoxemia (ie, increase in serum indoles, oxindoles, and other endotoxins) and eventually to systemic inflammation, are novel risk factors for the development of HE.[9]

Lactulose, a nonabsorbable disaccharide and a prebiotic, is currently used as a first-line agent for the treatment of HE. A standard oral dosage of 30 to 60 mL/d in 2 divided doses of lactulose has been shown to be effective in improving quality of life and cognitive functions in cirrhotic patients[10] and in reducing the prevalence of MHE.[11] Nevertheless, patient adherence to lactulose is poor because of its common adverse effects, mainly diarrhea, bloating, and flatulence.[12] Other potential options that could be considered for the treatment of HE are the use of the antibiotic rifaximin and L-ornithine-L-aspartate (LOLA). Oral rifaximin, especially in combination with lactulose, has been shown to reduce the recurrence of HE episodes and increase quality of life; however, it is costly and there remains a lack of evidence supporting its use as a monotherapy for preventing recurrence of HE.[13,14] The administration of LOLA has shown positive effects in reducing ammonia levels[15] but further high-quality studies are needed to assess its efficacy, tolerance, and cost-effectiveness.

Probiotics (eg, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*) are live beneficial bacteria which, when ingested, may confer a health benefit on the host.[9] Prebiotics (eg, lactulose and fructooligosaccharides, mainly inulin) are "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, and thus improve host health".[16] Symbiotics are the combination of prebiotics and probiotics. It has been suggested that prebiotics, probiotics, and symbiotics could be a potential therapy for HE because of their beneficial effects on modifying the gut microflora.[10]

Previous systematic reviews and meta-analyses have been conducted regarding the effect of probiotics[17] and/or symbiotics[18–20] versus placebo, no therapy and/or lactulose mainly on the improvement of HE and ammonia levels.

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More recent meta-analyses have concluded that probiotics and symbiotics appear to improve not only HE and ammonia levels but also quality of life, hospitalization rates, and mortality.[21,22] However, there have not yet been any publications reporting the effect of probiotics and symbiotics in cirrhotic patients on other novel risk factors for HE such as increase on beneficial bacteria and decrease of SIBO, inflammation, and endotoxemia and in comparison with other therapies such as rifaximin and LOLA. Therefore, the present systematic review aims to assess the effect of probiotics and/or symbiotics versus placebo, no therapy, lactulose, rifaximin, and/or LOLA in changes on intestinal microflora, and reduction of endotoxemia and inflammation as primary outcomes, as well as their effect on the reversal of MHE and the development of OHE, the impact on quality of life and the reduction of total ammonia as secondary outcomes.

MATERIALS AND METHODS

Literature Search

This systematic review was conducted according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[23]

Search Strategy

The databases PubMed, ISI Web of Science, EMBASE, and Cochrane Library were used for the searching of the literature relating to the effect of probiotics and symbiotics on HE in patients with cirrhosis. The search strategy included the following mix of keywords: "symbiotic" and "hepatic cirrhosis," "symbiotic" and "hepatic encephalopathy," "synbiotic" and "hepatic cirrhosis," "synbiotic" and "hepatic encephalopathy," "probiotic" and "hepatic cirrhosis," and "probiotic" and "hepatic encephalopathy." These terms were searched in titles and/or abstracts; when the abstract was not available, there was a revision of the full article.

Studies Selection

The criteria used to identify articles for inclusion in this systematic review were the following:

(a) Articles published in English.
(b) Type of study: randomized controlled clinical trials (RCTs).
(c) Type of study participant: adult (18 y or older) patients with hepatic cirrhosis.
(d) Exposure variable: use of probiotics and/or symbiotics in 1 arm and a comparative arm receiving placebo, no therapy, lactulose, rifaximin, or LOLA irrespective of the duration of the intervention.
(e) Primary outcomes: changes on intestinal microflora (ie, increase on beneficial bacteria and decrease of SIBO) and reduction of endotoxemia and inflammation.
(f) Secondary outcomes: reversal of MHE, development of OHE, decrease of serum concentration of total ammonia and improvement of quality of life.

The selection process of target trials was conducted in the first instance by the primary researcher (D.V.H.) but then 2 co-authors (A.A. and R.S.) cross-checked the selection process.

Data Extraction

Data regarding the following aspects were extracted: author and year of publication, characteristics of the population of study, sample size, duration of intervention, characteristics of the study groups (description of the intervention), attrition rates, and outcomes related with the effect of probiotics and/or symbiotics in changes on intestinal microflora, reduction of endotoxemia and inflammation, reversal of MHE, development of OHE, decrease of serum concentration of total ammonia, and improvement of quality of life.

Quality Assessment

All of the RCT included were assessed for risk of bias according to The Cochrane Collaboration's tool for assessing risk of bias[24] using the following domains: sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other potential threats to validity (eg, stopped early due to some data-dependent process or had extreme baseline imbalance). To classify the included trials as low, moderate, or high risk of bias, a score based on the domains of The Cochrane Collaboration's tool was used: 1 point was given to each "low-risk" category, 2 points to each "uncertain" category, and 3 points to each "high-risk" category. After summing the points of all 6 domains, trials were classified as low risk of bias if they had a score of 6 to 7, moderate risk of bias with a score of 8 to 9, and trials scoring ≥ 10 were considered at high risk of bias. The quality of a body of evidence was also assessed by using the grading of recommendations assessment, development and evaluation (GRADE) system. The quality of the evidence was based on the extent of risk of bias, inconsistency, indirectness, imprecision, and publication bias that existed for the evidence supporting the intervention. The quality of evidence was described as high, moderate, low, and very low.[25,26]

Statistical Analysis

The meta-analysis was performed using the Review Manager software (version 5.3.5, Cochrane Informatics & Knowledge Management Department, http://tech.cochrane.org/revman/download). Reversal of MHE and development of OHE were analyzed using estimation of risk ratio (RR) with 95% confidence interval (CI). The results were pooled using the Mantel-Haenszel random-effects model. Reduction of ammonia and inflammation were analyzed using weighted mean differences (WMD) with 95% CI and results were compared through the use of an inverse variance random-effects model. Statistical heterogeneity was evaluated with X^2 and I^2 statistics, where X^2 assesses whether observed differences in results are compatible with chance alone, whereas I^2 provides an estimate of the amount of variance across studies resulting from heterogeneity rather than chance. Substantial heterogeneity was defined as $> 50\%$.[27] A $P < 0.05$ was considered to be significant.

RESULTS

Literature Search

The selection process for the articles is shown in Figure 1. A total of 376 articles were automatically identified by applying the search keywords. Of these, 357 were excluded for not meeting the inclusion criteria: 20 articles were published in languages other than English; 300 articles were not reporting on RCT; 27 articles were of studies conducted with participants who did not have

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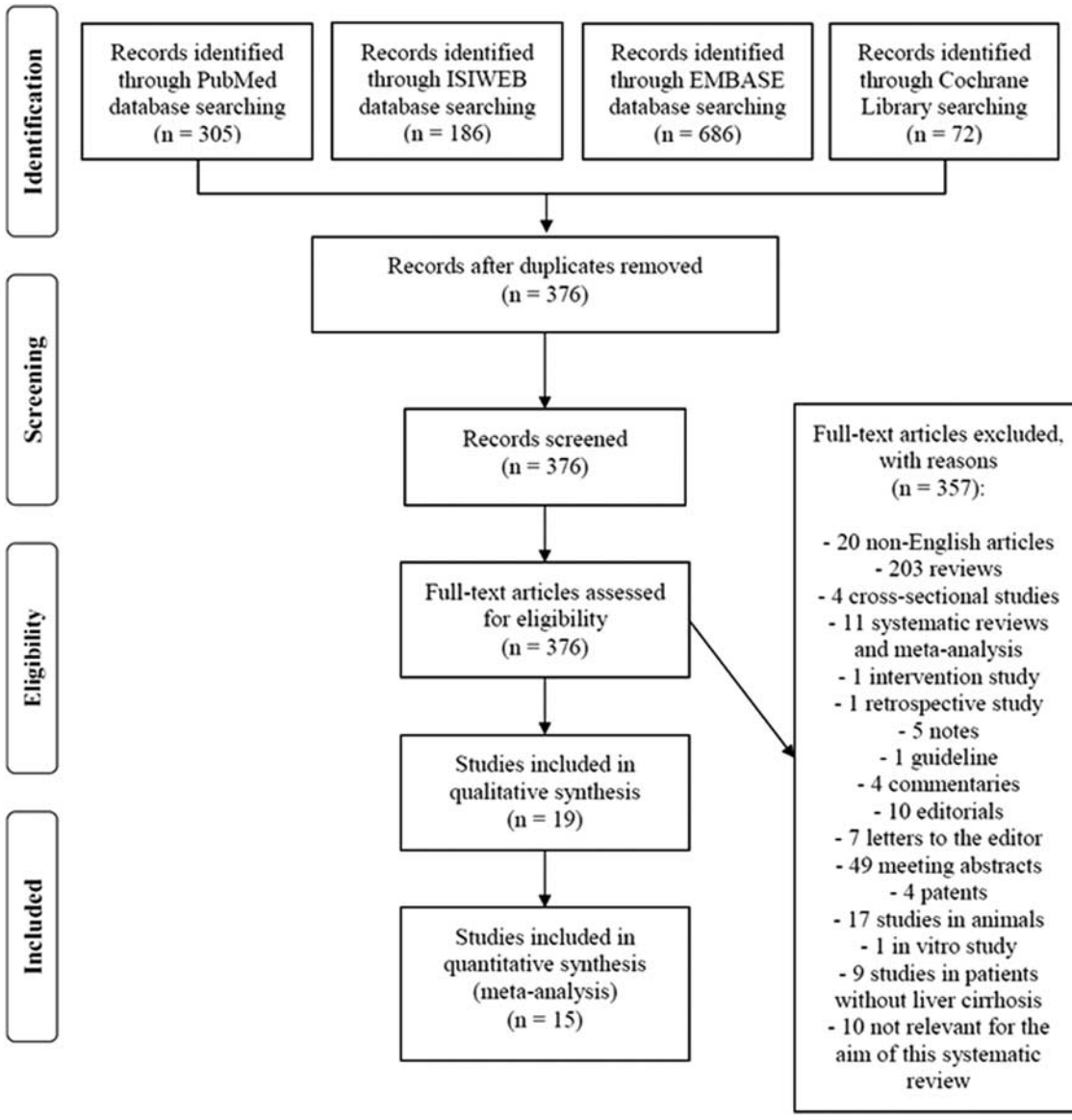

FIGURE 1. PRISMA flowchart showing study identification and selection process.

hepatic cirrhosis; and 10 articles reported on outcomes that were not relevant to the aim of the present systematic review (eg, hepatic and systemic hemodynamic alterations, hepatic venous pressure gradient, portal pressure, spontaneous bacterial peritonitis, liver function recovery, and neutrophil function). A total of 19 RCTs were included in this systematic review.[12,28–45]

Characteristics of the Trials and Participants

Nine trials investigated probiotics/symbiotics versus placebo/no treatment,[28,31–33,35–37,40,41] 6 investigated probiotics/symbiotics versus lactulose,[12,30,38,42,44,45] 2 investigated probiotics versus rifaximin,[29,34] 1 investigated probiotics versus rifaximin and LOLA,[43] and 1 looked at probiotics versus lactulose and LOLA.[39] The characteristics of the ~~RCT~~s included in this systematic review are presented in Table 1. The 19 eligible trials included a total number of 1668 participants and consisted of 7 trials in patients with cirrhosis,[28–30,33,34,36,40] 11 trials in patients with cirrhosis and MHE[12,31,32,35,37,39,41–45] and 1 trial in patients with cirrhosis, OHE (grades I and II), and hyperammonemia.[38] All of the trials included patients with a stable stage of cirrhosis as specified by their exclusion criteria [history or presence of gastrointestinal bleeding, infections, renal, heart and/or respiratory failure, electrolyte disturbances, hepatocellular carcinoma, neurological diseases (Alzheimer and/or Parkinson), spontaneous bacterial peritonitis, and inflammatory diseases]. Males formed the predominant patient population in all of the trials (n = 1234; 73.9%) except in 2 that did not mention the gender distribution.[31,40]

Quality Assessment

Table 2 shows the risk of bias assessment of the included RCTs. According to The Cochrane Collaboration's tool results, 4 trials scored 6 to 7 points and were

TABLE 1. Characteristics of the Randomized Controlled Clinical Trials Included in the Present Systematic Review

References	Population Study and Sample Size	Study Groups	Length of Treatment and Attrition Rates
Probiotics/symbiotics compared with placebo/no treatment			
Lata et al[28]	39 patients with cirrhosis Age (y): Intervention group: 53.2 (35-65) Control group: 51.4 (34-59) Male: 61.5% Female: 38.5%	Intervention group (n = 22): 1 capsule containing $2.5\text{-}25 \times 10^9$ CFU of *Escherichia coli* Nissle (Mutaflor) for the first 4 days, and after 5 days, 2 capsules were taken before breakfast. Control group (n = 17): capsules of placebo containing sucrose	42 days Total: 0.0% (0)
Bajaj et al[31]	25 nonalcoholic cirrhotic patients with MHE Age (y): Intervention group: 52.0 ± 8.0 Control group: 54.0 ± 4.0	Intervention group (n = 17): probiotic yogurt [*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Bifidobacterium*, and *Streptococcus thermophiles* (12 ounces/day)] Control group (n = 8): no treatment	2 mo Intervention group: 17.6% (3) Control group: 0.0% (0)
Pereg et al[40]	40 patients with cirrhosis Age (y): Intervention group: 63.2 ± 10.5 Control group: 65.9 ± 8.4	Intervention group (n = 20): capsules containing 4 freeze-dried bacteria (*L. acidophilus*, *L. bulgaricus*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*) each at a daily dose of 2×10^{10} CFU Control group (n = 20): capsules of placebo containing wheat-based nonfermentable fibers	6 mo Intervention group: 10.0% (2) Control group: 10.0% (2)
Saji et al[41]	43 patients with cirrhosis and MHE Age (y): Intervention group: 50.6 ± 5.8 Control group: 52.1 ± 10.1 Male: 92.5% Female: 7.5%	Intervention group (n = 21): 1 sachet containing 1.25×10^{12} spores of *L. acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Sacharomyces boulardi*, tid after meals Control group (n = 22): placebo powder in identical looking sachet, tid after meals	1 mo Intervention group: 4.7% (1) Control group: 9.0% (2)
Bajaj et al[32]	37 patients with cirrhosis and MHE Age (y): Intervention group: 56.3 ± 9.0 Control group: 58.4 ± 4.3 Male: 67.5% Female: 32.5%	Intervention group (n = 18): *Lactobacillus LG AT 53103* at a dose of 50×10^{12} CFU Control group (n = 19): placebo without probiotic	2 mo Intervention group: 16.6% (3) Control group: 15.7% (3)
Dhiman et al[33]	130 patients with cirrhosis Age (y): Intervention group: 48.0 (45.2-50.8) Control group: 50.1 (47.6-52.5) Male: 85.6% Female: 15.4%	Intervention group (n = 66): VSL#3, lyophilized probiotic preparation granulated powder with 4 *Lactobacillus* species (*L. paracasei*, *L. plantarum*, *L. acidophilus*, and *L. bulgaricus*), 3 *Bifidobacterium* species (*B. longum*, *B. infantis*, and *B. breve*) and *Streptococcus thermophilus* in a dose of 1 sachet/day with 9×10^{11} CFU per sachet Control group (n = 64): corn flour placebo	6 mo Intervention group: 40.9% (27) Control group: 28.1% (18)
Lunia et al[36]	160 patients with cirrhosis Mean age: 48.6 ± 11.1 y Male: 60.0% Female: 40.0%	Intervention group (n = 86): VSL#3 (*B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *S. thermophiles*); 3 capsules/day; total dose 1.1×10^{12} CFU Control group (n = 74): no treatment	3 mo Total: 6.9% (11)
Malaguarnera et al[37]	60 patients with cirrhosis and MHE Age (y): Intervention group: 46.0 ± 11.0 Control group:45.0 ± 12.0 Male: 55.0% Female: 45.0%	Intervention group (n = 30): *B. longum* + FOS Control group (n = 30): placebo (vitamins B_1, B_2, B_6, and B_{12})	3 mo Total: 0.0% (0)
Liu et al[35]	55 patients with cirrhosis and MHE Age (y): Group A: 55.0 ± 12.0 Group B: 53.0 ± 10.0 Group C: 57.0 ± 12.0 Male: 96.3% Female: 3.7%	Group A (n = 20): 1 sachet/day; symbiotic (4 freeze-dried bacteria (*Pediacoccus pentoseceus*, *Leuconostoc mesenteroides*, *L. paracasei*, and *L. plantarum*), each at a dose of 1×10^{10} CFU per sachet and 10 g of bioactive, fermentable fiber (β glucan, inulin, pectin, and resistant starch) Group B (n = 20): 1 sachet/day of only the bioactive, fermentable fibers described above Group C (n = 15): 1 sachet/day of a wheat-based, nonfermentable placebo	1 mo Total: 0.0% (0)

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TABLE 1. (*continued*)

References	Population Study and Sample Size	Study Groups	Length of Treatment and Attrition Rates
Probiotics/symbiotics compared with lactulose			
Agrawal et al[30]	235 patients with cirrhosis Age: 18-70 y Male: 84.6% Female: 15.4%	Group A (n = 80): 30-60 mL of lactulose; group B (n = 77): 3 capsules/day containing 112.5×10^{11} CFU/capsule of viable lyophilized bacteria (4 strains of *Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, and *L. bulgaricus*], 3 strains of *Bifidobacterium* (*B. longum*, *B. breve*, and *B. infantis*) and *S. thermophiles*; group C (n = 78): no treatment	12 mo Group A: 15.0% (12) Group B: 16.8% (13) Group C: 16.6% (13)
Ziada et al[45]	90 patients with cirrhosis and MHE Age (y): Group A: 48.8 ± 8.2 Group B: 50.3 ± 7.8 Group C: 51.2 ± 7.5 Male: 73.3% Female: 26.7%	Group A (n = 30): 30-60 mL of lactulose; group B (n = 30): *L. acidophilus* 1×10^6 CFU/capsule tid; Group C (n = 30): control	1 mo Group A: 6.7% (2) Group B: 3.3% (1) Group C: 0.0% (0)
Malaguarnera et al[38]	125 patients with cirrhosis, OHE (grades 1 and 2) and hyperammonemia Mean age: 50.1 ± 9.4 Male: 91.6% Female: 8.4%	Group A (n = 63; 31 OHE grade 1, 32 OHE grade 2): lactulose; Group B (n = 62; 31 OHE grade 1, 31 OHE grade 2): *B. longum* + FOS	2 mo Total: 0.0% (0)
Pratap Mouli et al[12]	120 patients with cirrhosis and MHE Age (y): Group A: 44.2 ± 10.4 Group B: 39.6 ± 11.4 Male: 91.6% Female: 8.4%	Group A (n = 60): lactulose 30-60 mL/d; group B (n = 60): VSL#3 (*B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *S. thermophiles*); 4 capsules/day; total dose 4.5×10^{12} CFU	2 mo Group A: 33.3% (20) Group B: 45.0% (27)
Sharma et al[42]	105 patients with cirrhosis and MHE Mean age: 42.2 ± 11.8 y Male: 75.2% Female: 24.8%	Group A (n = 35): lactulose 30-60 mL/d; group B (n = 35): lyophilized probiotics 1 capsule tid (each capsule: *Streptococcus faecalis*, 60 million; *Clostridium butyricum*, 4 million; *Bacillus mesentericus*, 2 million; and *Lactobacillus*, 100 million); group C (n = 35): 30-60 mL/d of lactulose + probiotics	1 mo Group A: 11.4% (4) Group B: 11.4% (4) Group C: 14.2% (5)
Shavakhi et al[44]	60 patients with cirrhosis and MHE Mean age: 38.4 ± 9.6 y Male: 80.0% Female: 20.0%	Group A (n = 19): lactulose 30-60 mL/d plus FOS + lyophilized probiotics [*Lactobacillus* strains (*L. casei*, *L. rhamnosus*, *L. acidophilus*, and *L. bulgaricus*)], *Bifidobacterium* strains (*B. breve* and *B. longum*) and *S. thermophiles* in a total of 1×10^8 CFU per capsule, twice a day; group B (n = 21): lactulose 30-60 mL/d + placebo; group C (n = 20): FOS + probiotics	10 wk Group A: 5.2% (1) Group B: 4.7% (1) Group C: 0.0% (0)
Probiotics/symbiotics compared with rifaximin and/or LOLA			
Lighthouse et al[34]	30 patients with cirrhosis Age range: 51-71 y Male: 56.6% Female: 43.4%	Group A (n = 10): rifaximin 400 mg tid for 2 wk; group B (n = 10): probiotic SCM-III (*L. acidophilus*, *Lactobacillus helveticus*, and *Bifidobacterium*) 10 ml tid for 2 wk; group C (n = 10): rifaximin 400 mg tid for 1 wk followed by SCM-III 10 ml tid for 5 wk	6 wk Total: 0.0% (0)
Hotten et al[29]	30 patients with cirrhosis Age range: 58-74 y Male: 63% Female: 37%	Group A (n = 10): 20 g of lactitol tid; group B (n = 10): 400 mg of rifaximin bid; group C (n = 10): SCM-III [*L. acidophilus, Bifidobacterium, L. bulgaricus* in an ion/vitamin/phytochemical extracts-enriched medium (microflorana-F)] 10 mL tid	3 wk Total: 0.0% (0)
Sharma et al[43]	124 patients with cirrhosis and MHE Mean age: 39.1 ± 12.8 y Male: 62.0% Female: 38.0%	Group A (n = 31): 18 g of LOLA tid; group B (n = 31): 400 mg of rifaximin tid; group C (n = 32): 5×10^{12} CFU of lyophilized probiotics (*L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. casei*, *B. longum*, *B. infantis*, *B. breve*, *Sacchromyces boulardi*, and *S. thermophiles*); group D (n = 30): placebo	2 mo Total: 16.1% (20)

TABLE 1. (*continued*)

References	Population Study and Sample Size	Study Groups	Length of Treatment and Attrition Rates
Mittal et al[39]	160 patients with cirrhosis and MHE Age (y): Group A: 41.2 ± 11.9 Group B: 43.8 ± 10.9 Group C: 44.2 ± 11.8 Group D: 42.1 ± 8.7 Male: 76.8% Female: 23.2%	Group A (n = 40): standard treatment for cirrhosis; group B (n = 40): lactulose, 30-60 mL/d; group C (n = 40): 1.10×10^{10} CFU of probiotics; group D (n = 40): 18 g of LOLA/day	3 mo Group A: 22.5% (9) Group B: 12.5% (5) Group C: 15.0% (6) Group D: 20.0% (8)

bid indicates twice per day; CFU, colony-forming units; FOS, fructo-oligosaccharide; LOLA, L-ornithine-L-aspartate; MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy; tid, 3 times a day.

considered as low risk of bias,[30,31,33,37] 3 trials were classified as having moderate risk of bias because they scored 8 points,[12,32,36] and 12 trials scored ≥ 10 points and were considered at high risk of bias.[28,29,34,35,38–45]

The GRADE assessment of the quality of a body of evidence is displayed in Table 3. The quality of evidence regarding the effect of probiotics versus placebo on the development of OHE was considered moderate and the quality of the evidence for the reversal of MHE when the interventions were probiotics versus placebo or lactulose was assessed as being very low.

The evidence was considered as being low quality for the reduction of inflammation (probiotics vs. placebo), the reversal of MHE (probiotics vs. LOLA) and the development of OHE (probiotics vs. lactulose). The evidence for the reduction of ammonia levels was considered very low quality when the interventions were probiotics versus placebo and symbiotics versus lactulose; low quality for probiotics versus lactulose; and moderate quality when the intervention was symbiotics versus placebo.

Primary Outcomes

It was not possible to conduct a meta-analysis on the reduction of endotoxemia and changes on intestinal microflora due to the clinical heterogeneity on the reporting outcomes.

Changes on Intestinal Microflora

Four trials[28,31,35,45] reported a statistically significant increase of the beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and 5 trials[28,31,35,36,45] reported a significant decrease of SIBO when probiotics, symbiotics, and lactulose were compared with placebo/no treatment. According to the trial of Ziada et al,[45] no significant differences were observed on the gut microbiota when probiotics and lactulose were compared; however, probiotics significantly decreased the total count of pathogenic bacteria (eg, *Bacteroides* and *Clostridium*) when compared with rifaximin.[29]

Reduction of Inflammation

Three trials evaluated the effect of probiotics on inflammation. The pooled result showed that probiotics did not demonstrate any significant effect on the reduction of tumor necrosis factor-α (RR, −1.32; 95% CI: −3.56, 0.93; $P = 0.25$) or interleukin-6 (RR, 2.15; 95% CI: −0.20, 4.50; $P = 0.07$) in comparison with placebo/no treatment.[31–33]

Reduction of Endotoxemia

In 3 of the 19 trials included in this review,[31,33,35] a significant decrease of endotoxemia was observed when either probiotics or symbiotics were used as the intervention compared with placebo/no treatment.

Secondary Outcomes

Reversal of MHE and Development of OHE

Figures 2 and 3 display the results of the meta-analysis on the improvement of MHE and the prevention of OHE, respectively. Six of 9 trials[31,35,36,39,43,45] showed a significant reversal of MHE when probiotics were used as the intervention compared with placebo/no treatment (RR, 1.53; 95% CI: 1.14, 2.05; $P = 0.005$); however, the 9 trials[31,33,35,36,39,40,42,43,45] evaluating the effect of probiotics versus placebo on the reversal of MHE had substantial and significant statistical heterogeneity ($I^2 = 59\%$; $P = 0.01$). Probiotics showed no significant improvement of MHE in comparison with lactulose (RR, 0.92; 95% CI: 0.72, 1.18; $P = 0.52$)[39,42,43,45] or LOLA (RR, 0.87; 95% CI: 0.57, 1.32; $P = 0.50$).[39,43] Only 1 trial[43] reported a significant difference when probiotics were compared with rifaximin in reversing MHE.

Probiotics significantly reduced OHE development (RR, 0.62; 95% CI: 0.48, 0.80; $P = 0.0002$) compared with placebo/no treatment.[33,34,36,39,42,46] In comparison with lactulose, probiotics had no significant effect on the prevention of OHE (RR, 1.25; 95% CI: 0.82, 1.89; $P = 0.30$).[16,33,42,46]

Reduction of Ammonia Levels

Figure 4 shows the results of the meta-analysis on the reduction of ammonia levels. Seven trials evaluated the effect of probiotics on ammonia levels compared with placebo/no treatment[30,31,33,36,39,40,45]; 2 assessed the effect of symbiotics versus placebo[35,37]; 4 compared the effect of probiotics and lactulose[12,30,42,45]; and 2 evaluated the effect of symbiotics versus lactulose.[38,42] Probiotics and symbiotics had no significant effect on ammonia levels when compared with placebo (WMD, −6.16; 95% CI: −15.57, 3.26; $P = 0.20$) and lactulose (WMD, −5.25; 95% CI: −14.36, 3.85; $P = 0.26$), respectively, but a reduction in

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AQ3 **TABLE 2.** Risk of Bias Assessment of the Included Randomized Controlled Clinical Trials

References	Sequence Generation	Allocation Concealment	Blinding	Incomplete Outcome Data Addressed	Free of Selective Reporting	Other Potential Threats to Validity	Total Score
Lata et al[28]	2	2	2	2	2	2	12
Hotten et al[29]	2	2	2	2	1	2	11
Bajaj et al[31]	1	2	1	1	1	1	7
Pereg et al[40]	2	2	2	3	2	3	14
Saji et al[41]	1	2	2	3	2	2	12
Bajaj et al[32]	1	2	2	1	1	1	8
Dhiman et al[33]	1	1	1	2	1	1	7
Lunia et al[36]	1	1	1	3	1	1	8
Malaguarnera et al[37]	1	2	1	1	1	1	7
Liu et al[35]	3	2	2	2	2	2	13
Agrawal et al[30]	1	1	1	1	1	1	6
Ziada et al[45]	2	2	3	2	1	2	12
Pratap Mouli et al[12]	1	1	3	1	1	1	8
Sharma et al[42]	1	3	3	3	1	2	13
Malaguarnera et al[38]	1	2	2	2	2	2	11
Shavakhi et al[44]	1	2	3	2	1	2	11
Lighthouse et al[34]	2	2	3	1	1	2	11
Sharma et al[43]	1	2	3	2	2	2	12
Mittal et al[39]	1	2	3	2	2	2	12

1 = low-risk category; 2 = uncertain category; 3 = high-risk category.
Total score: 6 to 7 = low risk of bias; 8 to 9 = moderate risk of bias; ≥ 10 = high risk of bias.

ammonia levels in favor of probiotics was observed when compared with lactulose (WMD, −4.54; 95% CI: −9.53, 0.45; $P = 0.07$). Symbiotics significantly decreased ammonia levels when compared with placebo (WMD, −15.24; 95% CI: −26.01, −4.47; $P = 0.006$) but with substantial and significant heterogeneity ($I^2 = 83\%$; $P = 0.02$).

Improvement of Quality of Life

In 1 trial,[33] probiotics were shown to significantly improve the physical function and role physical domains and the physical component of the SF-36 quality of life questionnaire when compared with placebo. In addition, probiotics, lactulose, and LOLA significantly improved health-related quality of life versus no treatment, but no differences were observed between the 3 groups.[39]

Probiotics Versus Rifaximin: Effects on Ammonia and Endotoxin Levels

In 1 pilot trial,[34] 30 patients with cirrhosis were allocated by simple randomization to 3 groups: group A (rifaximin for 2 wk; n = 10), group B (probiotics for 2 wk; n = 10) and group C (rifaximin for 1 wk followed by probiotics for 5 wk; n = 10). Endotoxin and ammonia levels significantly decreased in groups A and B during the 2-week period treatment. After the suspension of treatment, endotoxin and ammonia levels showed a gradual increase in

TABLE 3. GRADE Assessment of the Quality of a Body of Evidence

Body of Evidence	Risk of Bias	Inconsistency	Indirectness	Imprecision	Quality of Evidence
Reversal of MHE					
Probiotics vs. placebo[31,33,35,36,39,40,42,43,45]	−2	−1	−1	+1	+1 (very low)
Probiotics vs. lactulose[12,39,42,45]	−2	0	−1	0	+1 (very low)
Probiotics vs. LOLA[39,43]	−1	−1	0	0	+2 (low)
Development of OHE					
Probiotics vs. placebo[30,31,33,36,39,45]	−2	0	0	+1	+3 (moderate)
Probiotics vs. lactulose[12,30,39,45]	−1	−1	0	0	+2 (low)
Reduction of ammonia					
Probiotics vs. placebo[30,31,33,36,39,40,45]	−2	−1	0	0	+1 (very low)
Symbiotics vs. placebo[35,37]	−1	−1	0	+1	+3 (moderate)
Probiotics vs. lactulose[12,30,42,45]	−2	0	0	0	+2 (low)
Symbiotics vs. lactulose[38,42]	−2	0	−1	0	+1 (very low)
Reduction of inflammation					
Probiotics vs. placebo[31–33]	−1	−1	0	0	+2 (low)

As all of the included studies were randomized controlled clinical trials, initial score was +4.
GRADE indicates grading of recommendations assessment, development and evaluation; LOLA, L-ornithine-L-aspartate; MHE, minimal hepatic encephalopathy; OHE, overt hepatic encephalopathy.

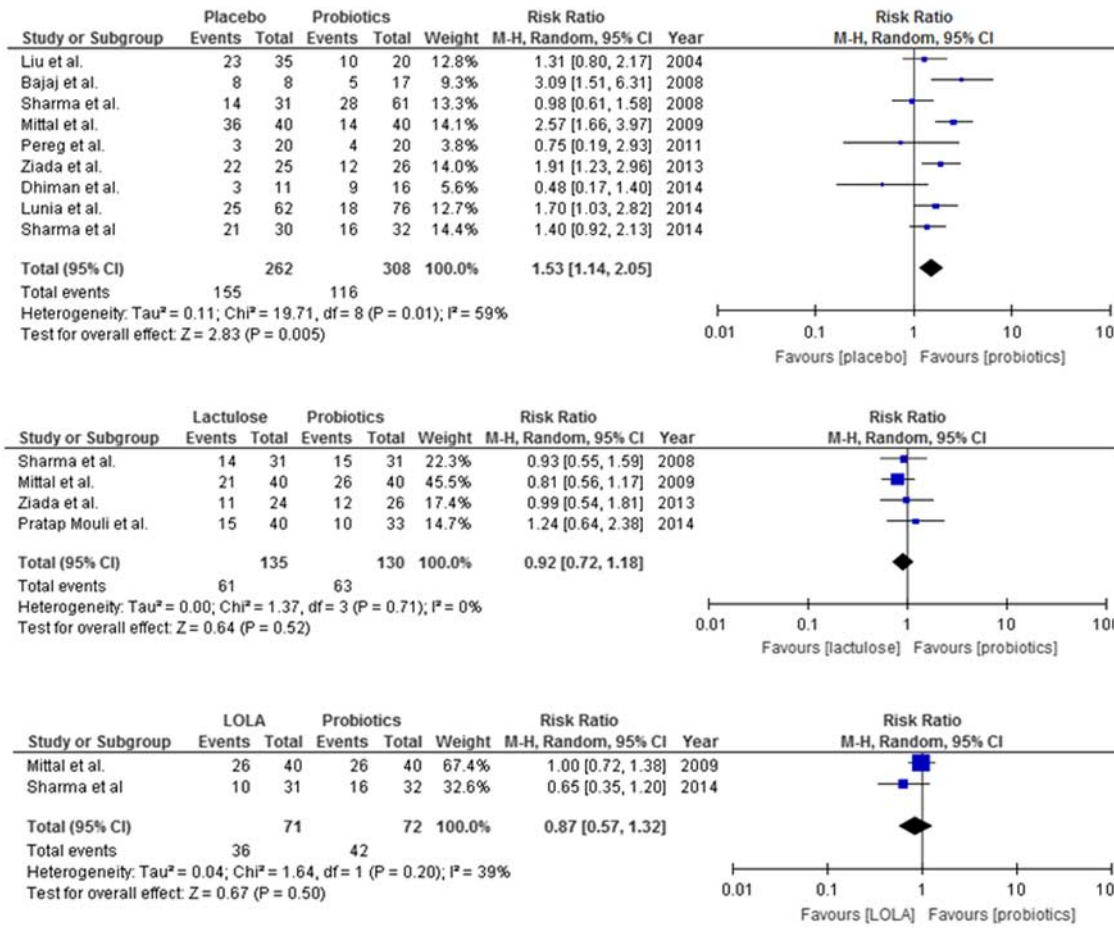

FIGURE 2. Forest plot displaying the effect of probiotics versus placebo, LOLA on the reversal of minimal hepatic encephalopathy. CI indicates confidence interval; LOLA, lactulose or L-ornithine-L-aspartate. full color online

groups A and B, but this increase occurred earlier in group A for endotoxin levels. Group C experienced the most significant long-standing normalization of endotoxin and ammonia levels (~~Fig. 5~~).

DISCUSSION

The results of the present review suggest that therapy with probiotics and symbiotics significantly decreases endotoxemia and changes the alterations of intestinal microflora by increasing the counts of beneficial bacteria and decreasing SIBO compared with placebo/no treatment. This review also showed that probiotics were as good as lactulose in changing the gut microbiota (ie, increase on beneficial bacteria and decrease of SIBO), reversing MHE, preventing the development of OHE and reducing ammonia levels. When meta-analyses were able to be performed, it was reported that probiotics reverse MHE and prevent the development of OHE in comparison with placebo/no treatment. In addition, symbiotics significantly reduced ammonia levels compared with placebo/no treatment.

The intestinal overgrowth of pathogenic bacteria (ie, SIBO) increases the production and absorption of ammonia and endotoxins, which interact with Toll-like receptors leading to the activation of immune response and systemic inflammation.[9] High-serum concentrations of proinflammatory cytokines exacerbate the cerebral effect of ammonia,[47,48] which synergistically produce cognitive impairment and worsen the symptoms of HE. This interrelated process has been proposed as the possible leading culprit in the development of HE.[9] Probiotics and symbiotics have the beneficial effect of modulating the intestinal microflora through substrate deprivation for potentially pathogenic bacteria, have the ability to increase fecal ammonia nitrogen and pathogenic bacteria excretion and are consequently a potential therapy for HE.[9,16] In this systematic review, probiotics failed to demonstrate a beneficial effect on ammonia levels, which is in agreement with findings of previous systematic reviews[17,20]; conversely, symbiotics did reduce ammonia levels. Nevertheless, both bodies of evidence (ie, effect of probiotics or symbiotics on ammonia) showed statistical heterogeneity in effect sizes making it difficult to draw meaningful conclusions about the benefits of probiotics and symbiotics on ammonia levels. Even though probiotics have been shown to improve the intestinal permeability,[49] decrease the absorption of endotoxins[35] and, therefore, reduce local and systemic inflammation,[32] findings of the present meta-analysis showed that probiotics have no significant effect on the reduction of proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 compared with placebo/no treatment; this might be attributed to the small sample sizes of the included

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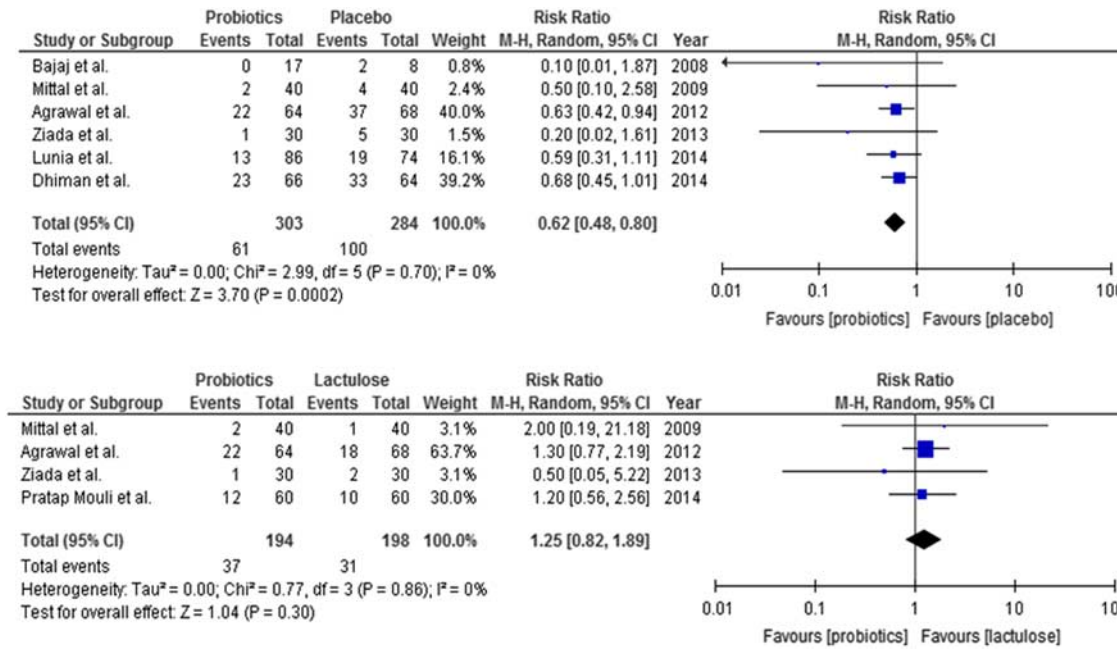

FIGURE 3. Forest plot displaying the effect of probiotics versus placebo or lactulose on the development of overt hepatic encephalopathy. CI indicates confidence interval. full color online

trials in the meta-analysis, which were underpowered to detect statistically significant differences. The significant reduction of SIBO and endotoxemia using either probiotics or symbiotics reported by 6 trials in the present systematic review[28,31,33,35,36,45] might partially explain the positive effects observed on the reversal of MHE and the prevention of the development of OHE.

Lactulose has for many years been considered the mainstay treatment for HE. It is effective in decreasing ammonia synthesis and absorption in the gut by acidifying the colonic lumen. This is as a result of the production of organic acids by bacterial fermentation (prebiotic effect), which increases fecal weight and shortens gastrointestinal transit time,[10] and also modulates the gut microflora by reducing pathogenic bacteria such as *Clostridium* and increasing acid-producing bacteria such as *Bifidobacterium* and *Lactobacillus*.[49] However long-term adherence to treatment with lactulose is very difficult to achieve because of its common side effects. In this systematic review, 24.4% of the patients included in the lactulose groups experienced diarrhea, bloating, flatulence, nausea, unpleasant taste, abdominal pain, and cramping,[12,30,38,44,45] whereas only few patients included in the probiotics groups (6.8%) experienced mild bloating.[12,30,44] Probiotics had fewer side effects than lactulose which might improve patient's compliance; however, there is still need of further research to confirm this.

The administration of LOLA in cirrhotic patients for the treatment of HE has been considered due to its efficacy in reducing ammonia levels.[15,46] Eight RCTs have demonstrated that LOLA improves both MHE and OHE by decreasing serum ammonia concentrations compared with the placebo/control groups.[46] However, later increases in ammonia levels appear to occur once treatment with LOLA is discontinued.[10] Few studies have compared the benefits of LOLA against other therapies for HE such as lactulose[50] and probiotics.[39,43] In the present systematic review, probiotics showed similar efficacy as LOLA in reversing MHE, but this should be interpreted with caution due to the small sample sizes, short length of treatment, and low quality of the included trials.[39,43] Consequently, future high quality and larger studies are needed to evaluate the efficacy of both probiotics and LOLA in improving HE.

Rifaximin, a broad-spectrum antibiotic that acts against pathogenic ammonia-producing enteric bacteria, is the first antibiotic to be licensed as maintenance treatment for HE and has been proven to be safe, well-tolerated, and effective in reducing the recurrence of OHE and HE-related hospitalizations.[51] Nevertheless, long-term treatment with rifaximin is markedly expensive [eg, 550 mg twice daily per year ($5250)] in comparison with other therapies used in clinical practice [eg, 1-year treatment with lactulose (30 to 60 mL/d) is $236 and with LOLA (500 mg/d) is $490] and probiotics (eg, 1-year treatment with probiotic VSL#3 is $900). In the reviewed trials, probiotics had a statistically significant effect on the reduction of pathogenic bacteria (ie, SIBO) in comparison with rifaximin,[29] but no significant differences regarding the reversal of MHE[43] and reduction of ammonia and endotoxin levels[34] were observed. Thus, further studies with longer follow-ups and bigger sample sizes are needed to evaluate and confirm the efficacy and cost-effectiveness of probiotics compared with rifaximin in the treatment of HE.

The optimal dose [colony forming units (CFU)], the method of delivery and the different probiotic species (use a single or a combination of probiotics) are the main topics regarding the use and effectiveness of probiotics that remain inconclusive. It is currently very difficult to interpret data from clinical trials using probiotics as the intervention, not only for the treatment of HE but also for other gastrointestinal diseases. The optimal number of CFU to claim a beneficial effect of probiotics is not yet known. Most of the clinical trials conducted in humans have used doses based on animal studies.[52] In the present review, all of the

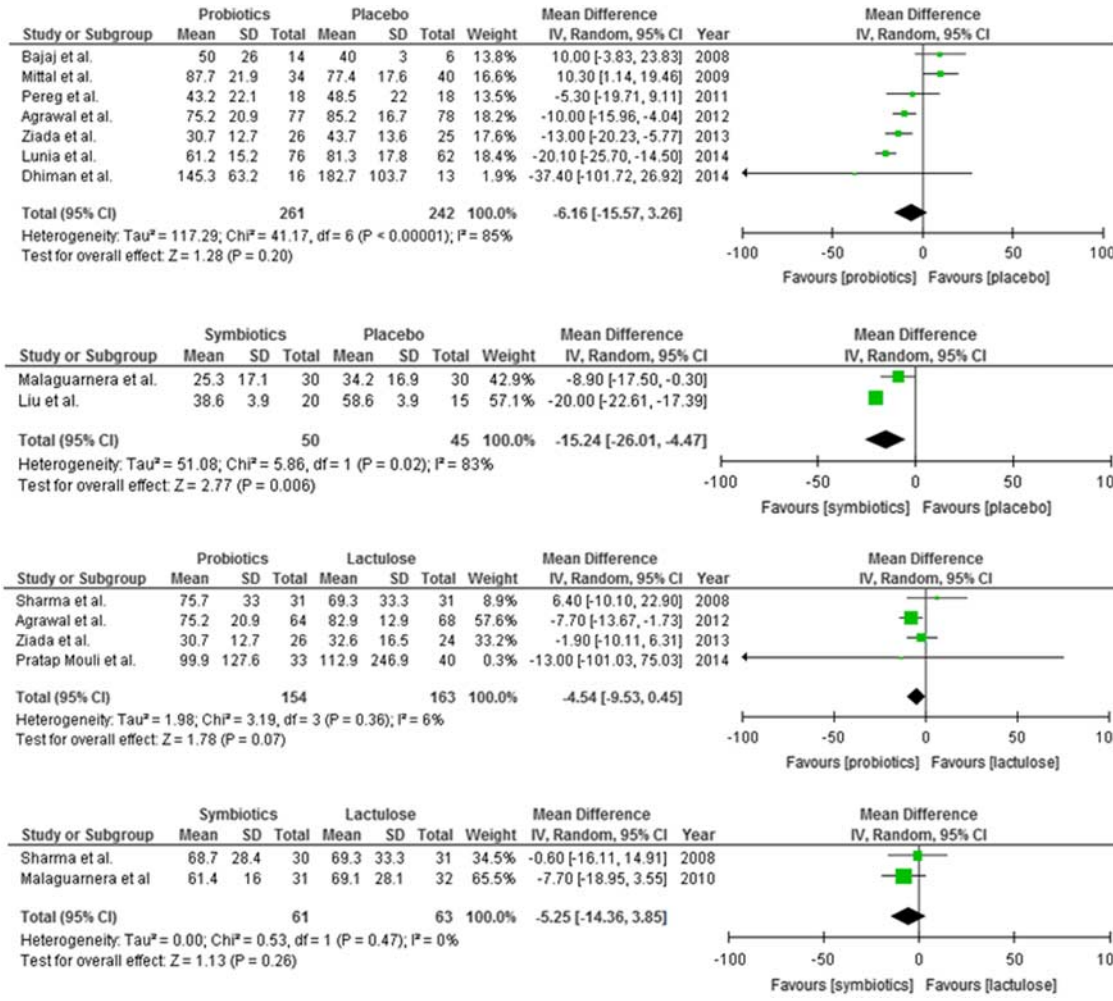

FIGURE 4. Forest plot displaying the effect of probiotics and symbiotics versus placebo or lactulose in the reduction of ammonia levels. CI indicates confidence interval. full color online

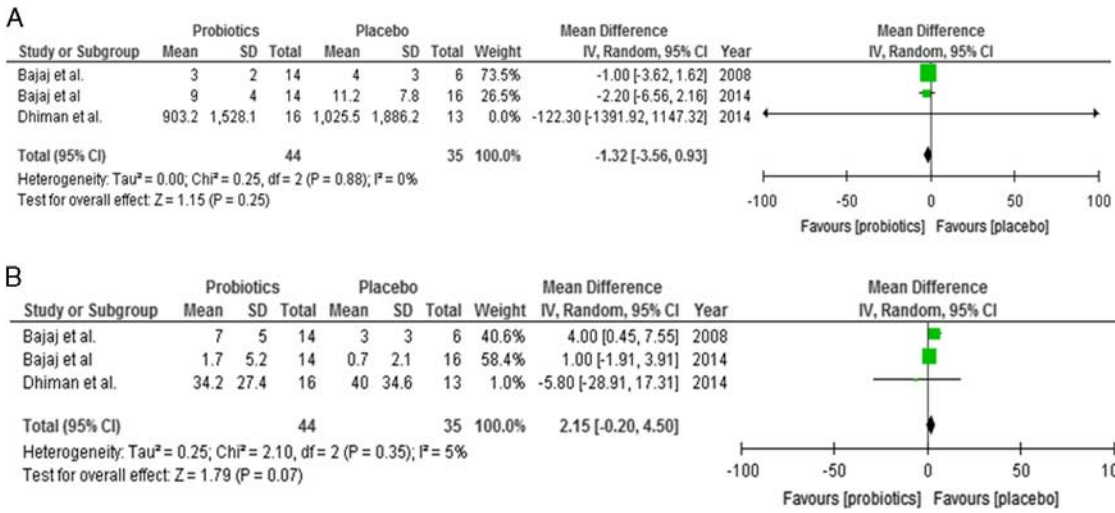

FIGURE 5. Forest plot displaying the effect of probiotics versus placebo on the reduction of inflammation. A, Reduction of tumor necrosis factor-α. B, Reduction of interleukin-6. CI indicates confidence interval. full color online

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included studies used different doses of CFU, which range from 1×10^6 to 50×10^{12} CFU. Although positive results were seen on gut microflora, endotoxemia, and HE in studies using doses from 1×10^6 CFU[45] to 5×10^{12} CFU,[43] no significant changes in HE were observed in 2 studies using a considerable number of CFU (1.25×10^{12} CFU[41] and 50×10^{12} CFU.[32])

The method of delivery (yogurt vs. freeze-dried/lyophilized bacteria) may have an impact on the survivability rates when passing through stomach acid and also on the viability to colonize the intestine.[52] In this review, all of the studies delivered probiotics in a freeze-dried state, except one that used a commercial yogurt (not currently available)[31]. However, only 4 studies[28,32,35,45] reported a significant increase in the gut content of beneficial bacteria, mainly *Lactobacillus* and *Bifidobacterium* species; therefore, it cannot be assumed that probiotics survived and were able to colonize the gut in the other studies.

Not all probiotics have the same properties; certain species may offer different immunological and physiological effects.[52] Only 5 of the studies included in this review used single-probiotic species: *Bifidobacterium longum*,[37,38] *Lactobacillus LG AT*,[32] *Escherichia coli* Nissle,[28] and *Lactobacillus acidophilus*,[45] whereas the remaining studies included a combination of different probiotic species, mainly *Lactobacillus*, B*ifidobacterium*, and *Streptococcus*; consequently, the significant changes observed in the single-probiotic studies can be attributed to those particular species, whereas in the rest of the studies the combination of different probiotics species might have had a synergistic beneficial effect on the host gut microflora.

The main strengths of the present systematic review and meta-analysis are as follows: (1) the literature search was conducted according to the PRISMA guidelines,[23] which helped to improve the clarity and transparency of the systematic review; and (2) the bibliographic searching was performed in 4 different databases with specific and well-defined keywords and therefore it was considered an extensive and complete search. However, there are some limitations that need to be highlighted: (1) the methodological quality of most of the included ~~RCT~~, was far from optimal. Lack of blinding, incomplete outcome data addressed and inconsistency (ie, statistical heterogeneity) were the quality assessment categories that contributed the most to this and influenced the results of the meta-analyses that were able to be performed; (2) the follow-up of almost all of the included trials was relatively short, which may limit the assessment of certain outcomes such as reversal of MHE and reduction of chronic inflammation, because both conditions have a more subtle course; and (3) sample sizes were small in the included trials, which also influenced the results of the possible meta-analysis performed and therefore caution should be taken when extrapolating the data to the general population with cirrhosis. Nevertheless, this is the first systematic review that provided for the first time valuable information regarding the efficacy of probiotics in improving the intestinal microflora, endotoxemia, and inflammation in patients with hepatic cirrhosis, which may have clinical significance in supporting this population.

In conclusion, findings of the present systematic review and meta-analysis have important clinical and research implications because they suggest that either probiotics or symbiotics could be an effective and well-tolerated alternative or complementary treatment to the standard therapy with lactulose to change the intestinal microflora and reduce endotoxemia and ammonia levels, and consequently to reverse MHE and prevent the development of OHE in patients with a stable stage of cirrhosis. Nevertheless, this review highlights the need for larger scale and high-quality RCTs with longer follow-ups to investigate the effect of probiotics and symbiotics in changes on gut microbiota, reduction of endotoxemia, inflammation and ammonia, prevention and/or reversal of HE, and improvement of quality of life. In addition, further research is needed to evaluate the efficacy, cost-effectiveness, and tolerance of probiotics and symbiotics compared with rifaximin and LOLA in the treatment of HE to recommend their alternative use in clinical practice.

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