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SYSTEMATIC REVIEW

Effectiveness and safety of probiotics as adjuvants in the eradication of *Helicobacter pylori*. Systematic review and meta-analysis

Efectividad y seguridad del uso de probióticos como adyuvantes en la erradicación de Helicobacter pylori. Revisión sistemática y metaanálisis

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Abstract

Introduction: Adding probiotics to triple and quadruple therapies has been proposed to improve their effectiveness in the eradication of *Helicobacter pylori,* but there is controversy about their usefulness.
Objective: To assess the effectiveness and safety of probiotics as adjuvants in triple or quadruple therapies for *H. pylori* eradication in adults.
Materials and methods: Systematic review and meta-analysis. Randomized clinical trials (RCTs) assessing the effectiveness and safety of probiotics as adjuvant therapy in combination with triple and quadruple therapies for the eradication of *H. pylori* in adults and published in English or Spanish between January 2010 and May 2020 were searched in the Embase, Ovid Medline, Cochrane Library, and LILACS databases. Regarding the meta-analysis, a fixed-effects model was used to calculate the pooled measure (OR and RR) of the effectiveness and safety of using adjuvant probiotics in triple and quadruple therapy.
Results: Twelve RCTs were included (1 091 patients in total): 9 assessed triple therapy, 2 assessed quadruple therapy, and 1 assessed both triple and quadruple therapy. In the case of triple therapy, the use of adjuvant probiotics was more effective than placebo: 79.4% vs. 71.1%, (OR=1.42; 95%CI: 1.05-2.09), but in the case of quadruple therapy, their use did not increase effectiveness. The most widely used probiotic was *Lactobacillus reuteri,* with an eradication rate of 77.9% (95%CI: 70.5-84.19) versus 66.8% (95%CI: 58.8-74.2) for placebo. Probiotics decreased the occurrence of adverse effects in both triple therapy (OR=0.50; 95%CI: 0.28-0.90) and quadruple therapy (OR=0.26; 95%CI: 0.09-0.74).
Conclusions. Adjuvant probiotics improve the effectiveness of triple therapy to eradicate *H. Pylori* by 8.5%, but the final effectiveness is <90%. Furthermore, their use does not increase the effectiveness of quadruple therapy. However, the use of these microorganisms reduces the adverse effects of these therapies.

Resumen

Introducción. Se ha propuesto agregar probióticos a las terapias triples y cuádruples para mejorar su efectividad en la erradicación de *Helicobacter pylori,* pero existe controversia sobre su utilidad.
Objetivo. Evaluar la efectividad y seguridad del uso adyuvante de probióticos en la terapia triple o cuádruple para la erradicación de *H. pylori* en adultos.
Materiales y métodos. Revisión sistemática y metaanálisis. Se realizó una búsqueda en Embase, Ovid Medline, Cochrane Library y LILACS de ensayos clínicos aleatorizados (ECA) publicados en inglés o español entre enero de 2010 y mayo de 2020 que evalúan la efectividad y seguridad de usar probióticos como terapia coadyuvante en combinación con la terapia triple o cuádruple en la erradicación de *H. pylori* en adultos. En el metaanálisis se utilizó un modelo de efectos fijos para calcular la medida combinada (OR y RR) de efectividad y seguridad de los probióticos coadyuvantes en terapia triple y cuádruple.
Resultados: Se incluyeron 12 ECA (1 091 pacientes en total): 9 evaluaron terapia triple; 2, terapia cuádruple, y 1, terapia triple y cuádruple. En la terapia triple el uso coadyuvante de probióticos fue más efectivo que el uso de placebo: 79.4% vs. 71.1% (OR=1.42; IC95%: 1.05-2.09), pero en la terapia cuádruple, su uso no aumentó la efectividad. El probiótico más utilizado fue *Lactobacillus reuteri,* con una tasa de curación de 77.9% (IC95%: 70.5-84.19) versus 66.8% (IC95%: 58.8-74.2) del placebo. Los probióticos disminuyeron la ocurrencia de efectos adversos tanto en terapia triple (OR=0.50; IC95%: 0.28-0.90) como en cuádruple (OR=0.26; IC95%: 0.09-0.74).
Conclusiones. El uso coadyuvante de probióticos mejora la efectividad de la terapia triple para erradicar *H. Pylori* en un 8.5%, pero la efectividad final es <90%. Además, su uso no aumenta la efectividad de la terapia cuádruple. No obstante, el uso de estos microorganismos disminuye los efectos adversos de estas terapias.

Introduction

Helicobacter Pylori is one of the most common bacterial infections worldwide, although it should be noted that most of the patients infected do not develop clinical manifestations of the disease.[1]

In a study on the origin of this bacterium, Linz *et al.*[2] describe that simulations indicate that *H. pylori* may have spread from East Africa about 58 000 years ago. Previously known as *Campylobacter pyloridis,* this microorganism was first described in 1982 by Marshall & Warren[3] in patients with chronic gastritis, and since then it has been reported to occur in more than 50% of the world's population, being less frequent in developed countries.[4,5] Currently, *H. pylori* is the main cause of chronic gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma (gastric MALT lymphoma), and gastric adenocarcinoma,[6-9] and is considered a type 1 carcinogen.[10]

H. pylori infection is usually acquired during childhood and persists unless eradicated with antibiotics,[6,8] so clinical consensus suggests that eradication should be pursued once it has been identified.[11-14] The treatment for the eradication of this bacterium involves the use of triple or quadruple therapy. The former combines a proton pump inhibitor (PPI) with two antibiotics, namely, amoxicillin and clarithromycin if *H. pylori* resistance is <15% or amoxicillin and metronidazole if *H. pylori* resistance is <45%,[11-13] which is recommended as initial treatment for 14 days.[11,12] The latter combines a PPI with two antibiotics plus bismuth subsalicylate in 14-day schedules at varying doses and frequencies.

It has been suggested that the schemes chosen for the initial treatment of *H. pylori* infection should achieve an effectiveness of 95% on a per-protocol (PP) basis or at least 90% on an intention-to-treat (ITT) basis.[11-14] Nearly 20 years ago, triple therapy had an effectiveness of 90% or more, but today that effectiveness has declined to as low as 70%,[9] which has been attributed primarily to antibiotic resistance.

In the last decade, it has been proposed that probiotics may be used as adjuvants in the eradication of *H. pylori* and the reduction of adverse effects secondary to triple or quadruple therapy used in the treatment of *H. pylori* infection. Increased eradication effectiveness has been reported mainly when probiotics are used in triple therapy, with an increase of 9-11%;[15,16] however, the same effect has not been reported when probiotics are used in quadruple therapy.[17,18] Thus, it is evident that the impact of probiotics on the eradication of *H. pylori* varies depending on the type of therapy used, which also leads to heterogeneity in their implementation in clinical practice due to the lack of consensus on their use.[11,14]

In view of the foregoing, the objective of the present systematic review and meta-analysis was to assess the effectiveness and safety of probiotics as adjuvants in triple or quadruple therapy for the eradication of *H. pylori* in adults. Likewise, as a secondary objective, it was proposed to explore the sources of heterogeneity that could explain the variable effect of probiotics depending on the therapy used that has been reported in the literature.

Materials and methods

Systematic review and meta-analysis aimed at answering the question: What is the effectiveness and safety of adjuvant probiotics in triple or quadruple therapy for the eradication of *H. pylori* in adults? This study followed the recommendations of the Cochrane Handbook for Systematic Reviews of Intervention[19] and the PRISMA criteria.[20]

Search strategy

A systematic search was performed in Embase, Ovid Medline, Cochrane Library and LILACS using the following strategy: type of study: randomized clinical trials (RCTs) assessing the effectiveness and safety of using probiotics as adjuvant therapy in

combination with triple or quadruple therapy for the eradication of *H. pylori* in adults; publication period: January 2010 to May 2020; language of publication: English and Spanish; search terms (alone and with different combinations and their variations depending on the database): "Helicobacter pylori", "H. pylori, eradication therapy", "triple therapy", "quadruple therapy, probiotics", "lactobacillus reuter", "Saccharomyces boulardii", "Lactobacillus plantarum", "Pediococcus acidilactici boulardii", "Lactobacillus rhamnosus", "Lactobacillus strains", "Lactobacillus bulgarius", "Streptococcus faecium", "Streptococcus thermophilus", and "Bifidobacterium longum", which were combined with the Boolean connectors "OR" and "AND" to establish the search equations.

Additionally, a gray literature search was performed in Google Scholar and the database search was supplemented by a snowball search.

Inclusion and exclusion criteria

Studies in adult patients (>18 years) in which the intervention group was treated with standard eradication therapy plus probiotics and the control group with the same eradication scheme with or without placebo were included. On the other hand, studies with pregnant women, trials using adjuvant eradication treatments other than probiotics, and papers whose full text was not available were excluded.

Two investigators (GJT and WAOR) independently performed the initial screening of the studies retrieved in the initial searches by reading titles and abstracts; subsequently, they verified compliance with the inclusion criteria by reading the full text. Disagreements regarding the final selection of studies were resolved by consensus.

Methodological quality and certainty of evidence

The methodological quality assessment of the selected studies was carried out based on the Cochrane Handbook for Systematic Reviews of Interventions,[19] which evaluates the following domains: random sequence generation (selection bias), allocation concealment (allocation bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), and selective reporting (reporting bias). The risk of publication bias was assessed using funnel plots. The GRADE approach was used to assess the degree of certainty of the evidence supporting the interventions.[21]

Data extraction

Two reviewers extracted the data from the studies included in the review (GJT and KEO). During this process, general information about the studies was obtained, such as author/authors, year and place of publication, design, and objectives. Likewise, the following information was extracted: characteristics and number of participants; types of standard therapy used (triple or quadruple); type of probiotics used; dose, frequency, and route of administration of probiotics; and quantitative data on the effectiveness and safety outcomes of adjuvant probiotics in combination with standard therapy for the eradication of *H. pylori*.

Statistical analysis

Data are presented descriptively using standardized tables, while information extracted from the studies included in the review is presented in summary tables. The pooled effect (odds ratio (OR) or relative risk (RR)) was estimated for the effectiveness (eradication)

and safety (adverse events) outcomes, with their respective confidence intervals (95%CI) depending on the type of outcome. Absolute risk reduction and relative risk reduction of the assessed interventions versus placebo or no intervention were also calculated.

The statistical model to determine the pooled effect using OR and RR was selected based on the number of included studies and their heterogeneity, which was tested during the analysis using the chi-square test (x^2) and the index of inconsistency (I^2), where heterogeneity values >30% measured with I2 but with *p*-values >0.05 obtained using x^2 showed that the differences in effect can be explained by chance. Given the foregoing, a fixed effects model for the meta-analysis and the Z value for the statistical significance test were performed to estimate the pooled measure of effect.

Subgroup analyses were also performed considering the type of eradication therapy (triple or quadruple) and the type of probiotic used. Moreover, clinical, statistical, and methodological sources of heterogeneity between studies were explored by means of a sensitivity analysis when heterogeneity according to I^2 was ≥60%. When it was not possible to explain the high heterogeneity (I^2≥60%) or when it was not possible to adjust by presenting the evidence by subgroups, the range of effects was presented per type of outcome based on the estimator reported in the studies, together with their respective 95%CI. Statistical analyses were performed in RevMan software.

Results

Identified and selected studies

The initial search yielded 1 023 results, 823 from the databases and 200 from Google Scholar, of which 367 were removed because they were duplicates and 578 after reviewing the title and abstract (504 and 74, respectively). Of the 78 references selected for full-text review, 21 were excluded because the full text was not available, 11 because they were written in languages other than English or Spanish, 15 because they were not RCTs, and 16 because they did not meet the inclusion criteria; there were also 3 duplicate studies (details of the excluded studies are presented in Annex 1). Thus, 12 studies were included for full analysis (Figure 1).

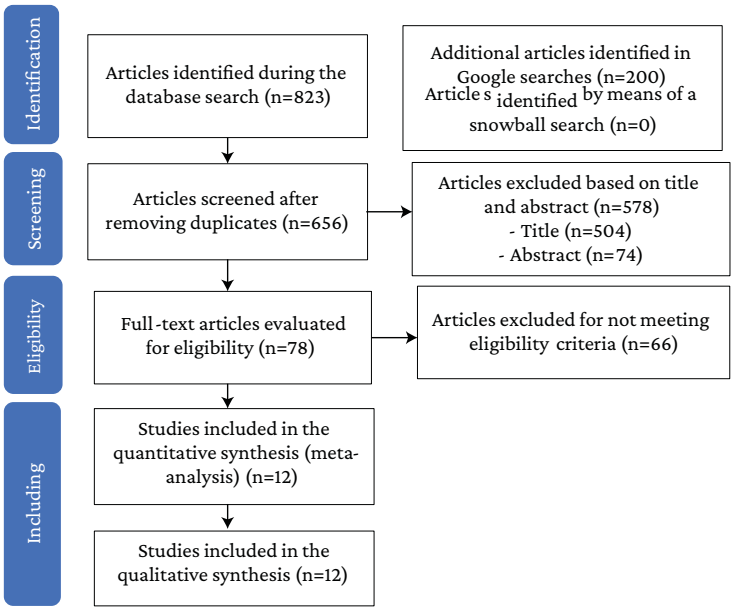

Figure 1. Flow chart of identified and selected studies.
Source: Own elaboration.

The 12 studies included in the meta-analysis are RCTs, and of these, 10 were double-blind,[22-31] 1 was triple-blind,[32] and in the other no placebo was used.[33] Triple therapy was used in 9 of these 12 studies[22-25,27-30,33] with a total of 824 participants, while quadruple therapy was used in 2,[31,32] with a total of 256 participants; similarly, one of these studies evaluated the use of both triple therapy and quadruple therapy.[26]

Studies on triple therapy

Of the 9 studies that used triple therapy, 2 used the drugs clarithromycin, amoxicillin, and lansoprazole.;[24,28] 6 used a triple therapy with different drugs: omeprazole plus clarithromycin and amoxicillin,[27] rabeprazole plus clarithromycin and tinidazole,[22] esomeprazole plus levofloxacin and amoxicillin,[33] lansoprazole plus tetracycline and furazolidone,[29] clarithromycin plus amoxicillin and rabeprazole,[30] and esomeprazole plus amoxicillin and clarithromycin;[23] finally, a single study used clarithromycin and amoxicillin and did not specify the PPI.[25]

The duration of triple therapy was variable: in 4 studies, probiotics were administered for 2 weeks;[24,27,30,33] in 4 studies, they were administered for 4 weeks;[22,23,28,29] and in 1 study, they were administered for 13 weeks.[25]

Studies on quadruple therapy

Both studies that analyzed quadruple therapy[31,32] used bismuth, amoxicillin, clarithromycin, and omeprazole for 2 weeks.[31,32]

Triple therapy and quadruple therapy studies

The study that evaluated the use of both therapies used omeprazole, clarithromycin and amoxicillin for 10 days in the triple therapy and bismuth, amoxicillin, clarithromycin and omeprazole, also for 10 days, in the quadruple therapy.[26]

Probiotics as an adjuvant therapy

Of the 12 studies included in the analysis, 4 used *Lactobacillus reuteri* as an adjuvant probiotic,[22,25,27,33] 5 used a mixture of probiotics,[26,28,29,31,32] 1 used *Saccharomyces*,[24] another used *Bacillus clausi*,[30] and another used *Bifidobacterium longum*.[23]

In 11 studies, a comparison versus placebo plus triple or quadruple therapy was made, except in the study by Ojetti *et al.*,[33] where the comparison was made only with triple therapy. Likewise, 10 studies specified that the placebo was equal to the investigational product in terms of form, presentation, smell and taste,[22-31] while the other did not specify anything in this regard.[32] Details of the included studies are presented in Tables 1 and 2.

Table 1. Overall characteristics of the included studies.

Study	Methods	Participants	Type of therapy	Intervention/dose/ duration	Control	Outcomes
Armuzzi *et al.*[22] 2001 Italy	Double-blind, prospective, randomized, placebo-controlled trial	Healthy asymptomatic patients over 18 years of age, positive for *Helicobacter pylori* and under treatment	Triple therapy: rabeprazole, clarithromycin and tinidazole	*Lactobacillus GG* for 14 days	Placebo	- Adverse events - Eradication
Chitapanarux *et al.*[23] 2015 Thailand	Double-blind, prospective, randomized, placebo-controlled trial	Patients with *H. Pylori* infection on antibiotic treatment	Triple therapy: esomeprazole, amoxicillin and clarithromycin	*Bifidobacterium longum* for 4 weeks	Placebo	- Adverse events - Eradication
Cindoruk *et al.*[24] 2007 Turkey	Prospective, randomized, placebo-controlled trial	Adult patients with *H. pylori* infection confirmed on upper digestive tract biopsy and dyspepsia symptoms	Triple therapy: clarithromycin, amoxicillin and lansoprazole	*Saccharomyces boulardii* for 14 days	Placebo	- Adverse events - Eradication
Francavilla *et al.*[25] 2014 Italy	Double-blind, prospective, randomized, placebo-controlled trial	Adult patients (over 18 years of age) with dyspepsia who had never received *H. pylori* eradication therapy	Triple therapy: proton pump inhibitor, clarithromycin and amoxicillin	*Lactobacillus reuteri* (mixture of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 6475) for 28 days	Placebo	- Adverse events - Eradication
Emara *et al.*[27] 2014 Egypt	Double-blind, prospective, randomized, placebo-controlled trial	Adult patients (18-60 years old) with dyspepsia and confirmed *H. pylori* infection and no previous treatment	Triple therapy: omeprazole, amoxicillin and clarithromycin	*L reuteri* (mixture of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 6475) for 4 weeks	Placebo	- Adverse events - Eradication
Myllyluoma *et al.*[28] 2005 Finland	Double-blind, randomized, placebo-controlled trial	Adult patients (18-70 years of age) with *H. pylori* infection confirmed by rapid blood test	Triple therapy: lansoprazole, clarithromycin and amoxicillin	*Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99, and *Propionibacterium freudenreichii* ssp. *shermanii* JS for 3 weeks	Placebo	- Adverse events - Eradication
Navarro-Rodríguez *et al.*[29] 2013 Brazil	Double-blind, randomized, placebo-controlled trial	Adult patients (over 18 years of age) with previously untreated *H. pylori* infection, diagnosed with peptic ulcer or functional dyspepsia, without decompensated chronic disease, and no use of anti-inflammatory drugs or antibiotics in the last 4 weeks	Triple therapy: lansoprazole, tetracycline and furazolidone	*Lactobacilos acidophilus, L. rhamnosus, Bifidobacterium bifidum*, and *Streptococcus faecium* for 30 days	Placebo	- Adverse events - Eradication
Nista *et al.*[30] 2004 Italy	Single-center, double-blind, prospective, randomized, placebo-controlled trial	Adult patients (18-65 years old) without gastrointestinal symptoms in the last 3 months and with *H. pylori* infection confirmed by breath test	Triple therapy: rabeprazole, clarithromycin, amoxicillin	*Bacillus clausii* and *Enterogermina* for 14 days	Placebo	- Adverse events - Eradication
Ojetti *et al.*[33] 2012 Italy	Single-center, controlled, prospective, randomized trial	Adult patients (18-65 years old) with *H. pylori* infection confirmed by breath test	Triple therapy: esomeprazole, levofloxacin and amoxicillin	*L. reuteri* for 14 days	None	- Adverse events - Eradication
Shafaghi *et al.*[31] 2016 Iran	Double-blind, prospective, randomized, placebo-controlled trial	Patients with *H. pylori* infection confirmed by histopathological examination	Quadruple therapy: bismuth, clarithromycin, amoxicillin and omeprazole	*Lactobacillus casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *B. longum*, and *Lactobacillus bulgarius* for 14 days	Placebo	- Adverse events - Eradication

Table 1. Overall characteristics of the included studies. (Continued)

Shavakhi *et al.*[32] 2013 Iran	Triple-blind, randomized, placebo-controlled trial	Adult patients with peptic ulcer disease and *H. pylori* infection confirmed by rapid urease test or histological studies	Quadruple therapy: omeprazole, bismuth, amoxicillin and clarithromycin.	Seven bacterial species including *Lactobacillus* strains *(L. casei, L. rhamnosus, L. acidophilus* and *L. bulgaricus*), *Bifidobacterium* strains (*B. breve* and *B. longum*), and *Streptococcus thermophiles*	Placebo	- Adverse events - Eradication
McNicholl *et al.*[26] 2018 Spain	Double-blind, randomized, placebo-controlled trial	Adult patients (18-70 years of age) with confirmed *H. pylori* infection	Triple therapy: omeprazole, clarithromycin and amoxicillin Quadruple therapy: clarithromycin, bismuth, amoxicillin and omeprazole	*Lactobacillus plantarum* CETC7879 and *Pediococcus acidilactici* CETC7880 for 10 days	Placebo	- Adverse events - Eradication

ATCC: American Type Culture Collection, Manassas, VA, USA. DSM: DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.
Source: Own elaboration.

Table 2. General characteristics of the studies.

Study	Number of patients	Probiotics	Control	Age (mean)	Female sex and probiotics	Female sex control (n)	Male sex and probiotics	Male sex control (n)	Eradication with probiotics	Eradication control (n)
Armuzzi *et al.*[22] 2001	60	30	30	40 years old	NR	NR	NR	NR	25	24
Chitapanarux *et al.*[23] 2015	63	31	32	NR	17	18	14	14	28	22
Cindoruk *et al.*[24] 2007	124	62	62	48 years old	36	44	26	18	44	37
Francavilla *et al.*[25] 2014	86	43	43	NR	32	29	18	21	33	29
McNicholl *et al.*[26] 2018	68	34	34	NR	NR	NR	NR	NR	32	31
Emara *et al.*[27] 2014	70	35	35	NR	22	24	13	11	26	23
Myllyluoma *et al.*[28] 2005	47	24	23	55.6 years old	13	16	10	8	21	19
Navarro-Rodríguez *et al.*[29] 2013	107	52	55	NR	34	33	21	19	45	40
Nista *et al.*[30] 2004	120	60	60	NR	27	35	33	25	39	37
Ojetti *et al.*[33] 2012	90	45	45	NR	NR	NR	NR	NR	36	27
Shafaghi *et al.*[31] 2016	76	38	38	43.5 years old	20	21	18	17	35	24
Shavakhi *et al.*[32] 2013	180	90	90	NR	41	30	49	60	73	69

NR: not reported; n: absolute number of participants.
Source: Own elaboration.

Bias assessment

The risk of random sequence generation (selection bias) was unclear in 3 studies.[22,30,31] In the allocation concealment domain, 2 studies had high risk[30,33] and the risk was unclear in 7.[22-24,26-28,31] In the domains blinding of participants and personnel, and blinding of outcome assessment, 1 study showed high risk[33] and in another the risk was unclear.[27] In the incomplete outcome data domain, 1 study showed high risk[23] and the risk was unclear in 2 studies.[30,31] Finally, in the selective reporting domain, the risk was not clear in 2 studies.[27,29] No other types of bias were observed in the studies (Figure 2).

Figure 2. Bias assessment of the included studies.
Source: Own elaboration.

The exploration of publication bias in the funnel plot is reported in Annex 2.

Outcomes

H. pylori eradication

The use of probiotics as adjuvant therapy in triple therapy for *H. pylori* eradication showed a benefit over the use of placebo (OR=1.48; 95%CI: 1.05-2.09; I^2=0%), as illustrated in Figure 3, which also reports the x^2 and I^2 heterogeneity values and the Z value of the statistical significance test for the pooled measure of effect.

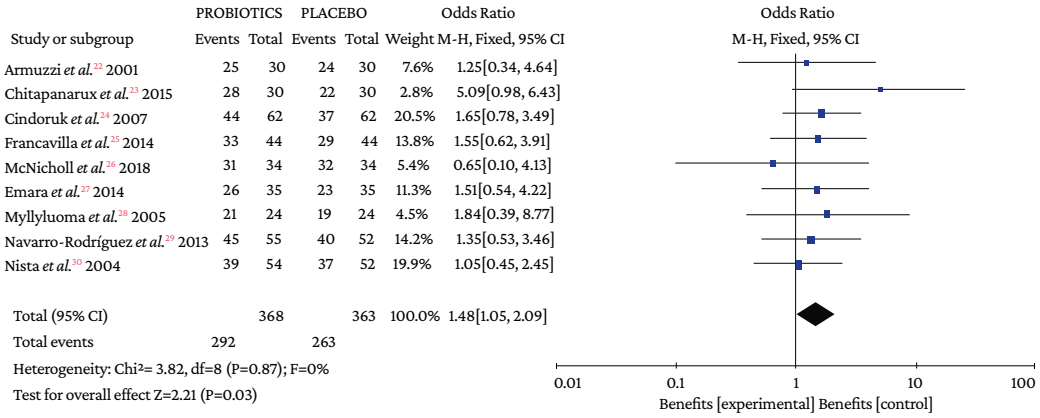

Figure 3. Pooled effect measure for *Helicobacter pylori* eradication outcome in triple therapy.
Events: number of *H. pylori* eradication events; Total: number of participants in each group (triple therapy plus probiotics is labeled as probiotics and triple therapy plus placebo as placebo).
Source: Own elaboration.

Regarding the use of adjuvant probiotics in quadruple therapy, it was not possible to estimate a pooled effect measure due to the high heterogeneity (I^2=78%); however, in the three studies identified, there was no evidence of differences in the effect found between the use of probiotics and placebo as adjuvant therapy: (OR=7, 95%CI: 0.35-138.14),[26] (OR=6.81, 95%CI: 1.76-26.27),[31] and (OR=0.77, 95%CI: 0.37-1.57).[32] Moreover, an absolute and relative increase in *H. pylori* eradication success with probiotics versus placebo of 8.3% and 11%, respectively, was found. Likewise, adjuvant therapy with the probiotic *L. reuteri* plus quadruple therapy showed a significant benefit compared to placebo (RR=1.75, 95%CI: 1.05-2.92; I^2=0 %), with an absolute eradication rate of the probiotic group exceeding the eradication treatment by 5.51% and a relative increase in eradication rate success of 14.16% (Annex 3).

In the relative risk analysis, it was observed that 2 studies using a probiotic mixture did not show a significant benefit in terms of effectiveness compared to the use of placebo: (RR=0.969, 95%CI: 0.847-1.108)[26] and (RR=0.945, 95%CI: 0.812-1.100)[32] (Table 3).

Table 3. Assessment by probiotic in triple and quadruple therapy.

Study	Relative risk reported in the original study	95%CI	Risk difference	95%CI
Probiotic mixture				
McNicholl *et al.*[26] 2018	0.969	0.847-1.108	-0.029	(-0.153)-0.094
Myllyluoma *et al.*[28] 2005	1.105	0.857-1.426	0.083	(-0.127)-0.293
Navarro-Rodríguez *et al.*[29] 2013	1.064	0.876-1.292	0.049	(-0.104)-0.202
Shafaghi *et al.*[31] 2016	1.458	1.124-1.891	0.289	0.114-0.465
Shavakhi *et al.*[32] 2013	0.945	0.812-1.100	-0.044	(-0.163)-0.075
Saccharomyces				
Cindoruk *et al.*[24] 2007	1.189	0.918-1.541	0.113	(-0.053)-0.279
Bacillus clausii				
Nista *et al.*[30] 2004	1.015	0.799-1.290	0.010	(-0.161)-0.182
Bifidobacterium longum				
Chitapanarux *et al.*[23] 2015	1.314	1.013-1.705	0.216	0.024-0.407

CI: confidence interval.
Source: Own elaboration.

Safety profile: adverse events

The use of probiotics in combination with triple therapy was associated with lower overall adverse effects compared to placebo (OR=0.50, 95%CI: 0.28-0.90; I^2=0%), which also occurred in combination with quadruple therapy (OR=0.26, 95%CI: 0.09-0.74; I^2=0%) (Figure 4).

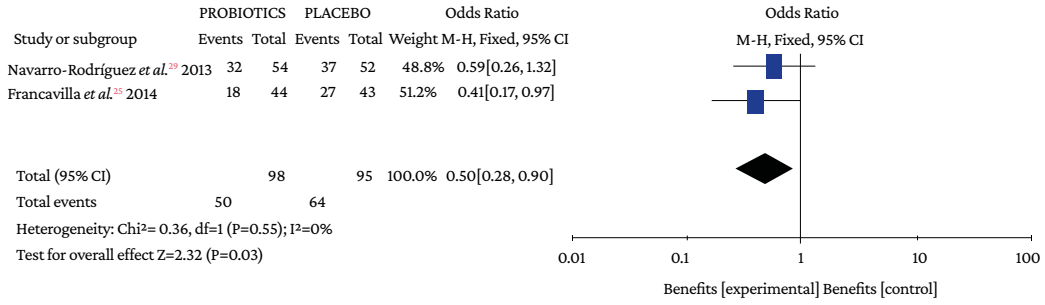

Figure 4. Adverse events with triple therapy.
Events: number of overall adverse events; Total: number of participants in each group (triple therapy plus probiotic is labeled as probiotics and triple therapy plus placebo as placebo).
Source: Own elaboration.

When analyzing the occurrence of adverse events in triple and quadruple therapy, it was found that the absolute effect of any adverse event in the probiotics group was lower by 16.4% and 9.37%, respectively. Likewise, the absolute and relative reduction in the risk of suffering any adverse event with probiotics plus triple therapy was 16.34% and 24.26%, respectively (Table 3).

Regarding the type of adverse event reported, no differences were found with the use of probiotics plus triple therapy compared to placebo in relation to abdominal pain (OR=0.57, 95%CI: 0.28-1.14; I^2=44%), anorexia (OR=0.63, 95%CI: 0.36-1.11; I^2=13%), or rash

(OR=0.64, 95%CI: 0.29-1.38; I^2=0%) (Annex 3). However, in the group with probiotics, a lower occurrence of pain in the epigastric region (OR=0.50, 95%CI: 0.33-0.77; I^2=0%), nausea (OR=0.32, 95%CI: 0.20-0.51; I^2=0%), diarrhea (OR=0.25, 95%CI: 0.16-0.39), and taste disturbance (OR=0.60, 95%CI: 0.40-0.91) was observed compared to the placebo group (Annex 3).

Assessment of evidence certainty

The evidence certainty assessment (GRADE approach) for patients receiving triple and quadruple therapy found that the certainty of the evidence evaluating the eradication of *H. pylori* with probiotic adjuvant triple therapy was moderate, while the certainty of the evidence for the occurrence of adverse events such as diarrhea, pain in the epigastric region, hyporexia, rash, among others, ranged from very low to moderate. In the case of *H. pylori* eradication with adjuvant probiotics in quadruple therapy, the certainty of the evidence was moderate, but when evaluating the decrease in adverse events such as diarrhea, the certainty of the evidence was high (Annex 4).

Discussion

In the present review, which looked into the effectiveness and safety of adding probiotics to conventional treatment for *H. pylori* eradication, it was found that, compared to the use of placebo, adding probiotics to triple therapy increased the eradication rate by 8.3%. This increase is similar to the one reported in other meta-analyses such as that of Gong *et al.,*[34] which included 23 randomized clinical trials involving 3 900 subjects receiving triple therapy and found that the addition of probiotics to this therapy increased eradication by 8.48% (72.26% vs. 80.74%); Zhang *et al.*,[35] which included data from 6 997 participants from 45 randomized controlled trials and showed that adding probiotics to standard therapy increased the eradication rate by 10.23% over placebo (82.31% vs. 72.08%); and the double-blind, randomized, placebo-controlled trial conducted by Dore *et al.*,[16] who analyzed 56 subjects with a low cure rate and found that the effectiveness rate in the probiotics group was 12.5% for PP and 10.7% for ITT. The meta-analysis carried out by Lau *et al.,*[15] in which 30 RCTs with a total of 8 817 patients were analyzed, also reported comparable results: the eradication rate increased by 12.2% when probiotics were added to triple therapy.

The present meta-analysis found that the most commonly used probiotic was *L. reuteri*, which was evaluated in 4 studies (154 patients in total).[22,25,27,33] The eradication rate with this microorganism was 77.9% (95%CI: 70.5-84.19), while placebo showed 66.8% (95%CI: 58.8-74.2), which is consistent with the results reported by Dore *et al.*[16] who compared the use of *L. reuteri* (Gastrus®) plus PPI versus placebo plus PPI, finding that the eradication of *H. pylori* was higher with the first combination (11% vs. 3.6%).

Although there was evidence of an increase in the effectiveness of *H. pylori* eradication with the use of probiotics in combination with triple therapy, the effectiveness of the intervention measured as eradication did not exceed 90% for ITT or 95% for PP, which are the minimum thresholds required for eradication therapies for this bacterium.[11-14] Therefore, using empirical triple therapies with or without probiotics to eradicate *H. pylori* is not justified.

Unlike triple therapies, the present meta-analysis found that adding probiotics to quadruple therapies does not increase the effectiveness to eradicate *H. pylori* compared to placebo, which is 87.7% vs. 82.8% (OR=2.67, 95%CI: 0.44-16.4%; I^2=78%). However, it

should be kept in mind that the low statistical power of the individual studies and the high heterogeneity among the included studies (I^2=78%) hindered a pooled analysis.

Another hypothesis that could explain why there was no increase in the effectiveness of quadruple therapy with bismuth is the effect that bismuth would have on inhibiting the growth of probiotics.[16] In this regard, Dore *et al.*[18] report, based on two randomized open pilot studies with 46 patients each, that a 10-day quadruple therapy (bismuth, teracycline, metronidazole, and PPI) had an effectiveness of 95.7% for PP (95% CI: 85-99%) and 84.8% for ITT (95% CI: 71-95%), and that patients treated with Gastrus® (BioGaia, Stockhplm, Sweden) showed an effectiveness of 79.6% for PP (95%CI: 72-97) and 84.8% for ITT (95%CI: 71-95). In the subgroup analysis, quadruple therapy with bismuth was 100% effective (25/25) in naive patients versus 90% (26/29) in those treated with Gastrus®. A drawback of the study by Dore *et al.*[18] is that it did not establish the susceptibility of *H. pylori* to the antibiotics used.

Concerning adverse effects, in the present study, an overall reduction of 16.34% was found for any adverse effect in triple therapies when probiotics were added, while the reduction in quadruple therapies was 9.37%. The individual analysis of adverse effects found that there was no difference between probiotics and placebo for the incidence of abdominal pain, anorexia or rash in triple therapies, although there was a lower incidence of pain in the epigastric region, nausea, diarrhea, and taste disturbance with the use of probiotics. However, it was not possible to determine whether the decrease in adverse effects improved treatment compliance.

Although there are other meta-analyses that assess the effectiveness of probiotics as an adjuvant to standard therapies for *H. Pylori* eradication, the present study has been conducted by subgroups of interest to establish the effectiveness of triple therapy plus probiotics when compared to triple therapy with placebo and no intervention, as well as the effectiveness of eradication therapy plus probiotics differentiated by type of probiotic, finding a moderate certainty of evidence to support this outcome, which ensures confidence in the results presented here.

In the case of quadruple therapy, this review has some limitations secondary to the low number of included studies comparing the adjuvant use of this type of therapy in combination with probiotics or placebo, which translates into a low statistical power that could not be corrected with pooled analyses given the high heterogeneity of the effects reported in these studies. In this regard, further studies are needed to evaluate whether adding probiotics to quadruple therapy increases its effectiveness in eradicating *H. pylori*.

Conclusion

The findings of the present study demonstrate that the adjuvant use of probiotics increases the effectiveness of triple therapy to eradicate *H. pylori* by 8.5%, but that the final effectiveness was not 90-95% in any case, which is the minimum threshold required in eradication therapies for this microorganism. Regarding quadruple therapy, it was established that probiotics do not modify its effectiveness, although there is uncertainty in the results given the inaccuracy of the effect estimators.

Concerning the safety of probiotics in combination with triple or quadruple therapy for *H. pylori* eradication, a lower incidence of adverse effects was found, although it could not be established whether this decrease favors adherence to treatment.

Based on the results obtained, it can be concluded that it is not necessary to continue studying the effect of adding probiotics to triple therapies for the eradication of *H. pylori*, but it is advisable to carry out new studies to evaluate this effect in quadruple therapies.

Explanatory note

This publication is derived from the thesis entitled *Efectividad y seguridad del uso de probióticos en la erradicación de Helicobacter pylori: revisión sistemática de la literatura y meta-análisis* (Effectiveness and safety of the use of probiotics in the eradication of *Helicobacter pylori*: systematic literature review and meta-analysis),[36] developed by the authors of this manuscript for obtaining a degree in gastroenterology.

Conflicts of interest

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Annex 1. List of excluded studies.

Reason for exclusion	No.	Article title	Author Year
Text not available/ abstract only	1	Therapeutic efficacy of pretreatment with compound acidophilus lactobacillus tablets followed by quadruple therapy in gastric ulcer patients with *Helicobacter pylori* infection	Wang & Shang 2019
	2	The influence of probiotics on the efficacy of eradication therapy of *Helicobacter pylori* infection	Mo 2019
	3	Probiotics improved the effectiveness and safety of the quadruple *Helicobacter pylori* eradication therapy	Jiang & Zhu 2018
	4	Can probiotics improve efficiency and safety profile of triple *Helicobacter pylori* eradication therapy? A prospective randomized study	Grgov *et al.* 2016
	5	Do probiotics improve eradication of *Helicobacter pylori* infection? A prospective study	Dajani 2015
	6	Probiotics in the treatment of peptic ulcer infected by *Helicobacter pylory* and its safety	Ma *et al.* 2015
	7	*H. Pylori* eradication rate is influenced by bacillus coagulans, lactoferrin and fructooligosaccharides and not by the dosage of proton pump inhibitors	D'Angelo *et al.* 2014
	8	Efficacy of probiotics in *Helicobacter pylori* eradication therapy	Yaşar *et al.* 2010
	9	Triple therapies plus different probiotics for *Helicobacter pylori* eradication	Scaccianoce *et al.* 2008
	10	The effects of probiotics on treatment of *Helicobacter pylori* eradication in children	Akcam *et al.* 2015
	11	Efficacy of *Helicobacter pylori* eradication taking into account its resistance to antibiotics	Ziemniak 2006
	12	Influence of *Clostridium butyricum* as probiotics supplementation on *Helicobacter pylori* eradication	Shimada *et al.* 2019
	13	New formulation of bismuth quadruple therapy for *Helicobacter pylori* eradication with and without probiotics: Efficacy and safety in daily clinical practice in Italy	Eusebi *et al.* 2018
	14	The effect of probiotics supplementation on *Helicobacter pylori* eradication rates and side effects during eradication therapy: a meta-analysis	Dang *et al.* 2014
	15	The impact of the probiotics of *Lactobacillus acidophilus* on the outcomes of *Helicobacter pylori* eradication treatment	Jang 2017
	16	The impact of probiotics on the outcomes of *Helicobacter pylori* eradication treatments	Seong *et al.* 2016
	17	Clinical outcomes and influencing factors of the standard triple therapy plus probiotics and concomitant therapy for first-line treatment of *Helicobacter pylori* infection	Cho *et al.* 2018
	18	The efficacy of probiotics As adjuvant treatment in eradicating *H. pylori* by standard triple therapy: A prospective, randomized, double blind and placebo-controlled trial	Hauser *et al.* 2014
	19	Control of *Helicobacter pylori* infection by dietary supplementation with *Lactobacillus reuteri* strain combination	Francavilla *et al.* 2013
	20	The effects of prebiotics and prebiotics during the anti-*Helicobacter pylori* triple therapy	Iakovlev *et al.* 2015
	21	Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori*?	Gotteland *et al.* 2006

Reason for exclusion	No.	Article title	Author Year
Full text not available in English or Spanish	1	[Influence of different timing of *Saccharomyces boulardii* combined with bismuth quadruple therapy for *Helicobacter pylori* eradication]	He *et al.* 2019
	2	Effects of addition of probiotic and/or bismuth to triple therapy of *H. pylori* and analysis of genetic variation of 23S rRNA gene between patients with clarithromycin sensitivity and resistance	Liu *et al.* 2019
	3	[Influence of two kinds of probiotics combined with bismuth quadruple therapy for *Helicobacter pylori* eradication]	Zhu *et al.* 2018
	4	Efficacy and safety of *Bifidobacterium* combined with ilaprazole-containning quadruple therapy in rescue eradication of *Helicobacter pylori*	Jin *et al.* 2017
	5	[Influence of *Saccharomyces boulardii* sachets combined with bismuth quadruple therapy for initial *Helicobacter pylori* eradication]	Zhu *et al.* 2017
	6	Effect of *Saccharomyces boulardii* on Rescue Therapy of *Helicobacter pylori* Infection	Lu *et al.* 2017
	7	[Clinical efficacy of bacillus subtilis combined with standard triple therapy for *Helicobacter pylori* infection]	Wang *et al.* 2017
	8	[Efficacy of levofloxacin-based triple therapy combined with probiotics as a rescue therapy for *Helicobacter pylori* re-eradication]	Peng *et al.* 2016
	9	[*Lactobacillus acidophilus* combined with quadruple therapy as a rescue therapy for *Helicobacter pylori* eradication]	Liu *et al.* 2013
	10	Therapeutic effect of *Saccharomyces boulardii* combined with standard triple therapy for *Helicobacter pylori* eradication	Gao *et al.* 2012
	11	[Effects of probiotic bifiform on efficacy of *Helicobacter pylori* infection treatment]	Iakovenko *et al.* 2006
Not a controlled clinical trial	1	The impact of *Helicobacter pylori* infection, eradication therapy and probiotic supplementation on gut microenvironment homeostasis: An open label, prospective clinical trial	Chen *et al.* 2018
	2	Efficacy of compound *Lactobacillus acidophilus* tablets combined with quadruple therapy for *Helicobacter pylori* eradication and its correlation with pH value in the stomach: A study protocol of a randomised, assessor-blinded, single-centre study	Ji *et al.* 2018
	3	High efficacy of 14-day standard triple therapy plus bismuth with probiotic supplement for *H. pylori* eradication in low clarithromycin resistance areas	Srinarong *et al.* 2014
	4	Improved eradication rate of standard triple therapy by adding bismuth and probiotic supplement for *Helicobacter pylori* treatment in Thailand	Srinarong *et al.* 2014
	5	Do probiotics improve eradication response to *Helicobacter pylori* on standard triple or sequential therapy?	Dajani *et al.* 2013
	6	Randomized control trial: Comparison of triple therapy plus probiotic yogurt vs. standard triple therapy on *Helicobacter pylori* eradication	Mirzaee & Rezahosseini 2012
	7	Evaluation of *Helicobacter pylori* eradication by triple therapy plus *Lactobacillus acidophilus* compared to triple therapy alone	Medeiros *et al.* 2011
	8	The effect of probiotics and mucoprotective agents on PPI-based triple therapy for eradication of *Helicobacter pylori*	Song *et al.* 2010
	9	Effects of multistrain probiotic-containing yogurt on second-line triple therapy for *Helicobacter pylori* infection	Yoon *et al.* 2011
	10	*Helicobacter pylori* and probiotics	Lesbros-Pantoflickova *et al.* 2007
	11	The effects of probiotics on PPI-triple therapy for *Helicobacter pylori* eradication	Kim *et al.* 2008

Reason for exclusion	No.	Article title	Author Year
Not a controlled clinical trial	12	Effect of *Lactobacillus casei* supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection	Tursi *et al.* 2004
	13	Pretreatment with *Lactobacillus*- and *Bifidobacterium*-containing yogurt can improve the efficacy of quadruple therapy in eradicating residual *Helicobacter pylori* infection after failed triple therapy	Sheu *et al.* 2006
	14	Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication	Sheu *et al.* 2002
	15	Kefir improves the efficacy and tolerability of triple therapy in eradicating *Helicobacter pylori*	Bekar *et al.* 2011
Criteria are not met	1	Effect of pretreatment with *Lactobacillus gasseri* OLL2716 on first line *Helicobacter pylori* eradication therapy	Deguchi *et al.* 2012
	2	A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates	Canducci *et al.* 2000
	3	Probiotic supplementation improves tolerance to *Helicobacter pylori* eradication therapy --a placebo-controlled, double-blind randomized pilot study	Myllyluoma *et al.* 2005
	4	Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: A parallel group, triple blind, placebo-controlled study	Cremonini *et al.* 2002
	5	Effect of pretreatment with *Lactobacillus delbrueckii* and *Streptococcus thermophillus* on tailored triple therapy for *Helicobacter pylori* eradication: A Prospective Randomized Controlled Clinical Trial	Tongtawee *et al.* 2015
	6	Effects of probiotics or broccoli supplementation on *Helicobacter pylori* eradication with standard clarithromycin-based triple therapy	Chang *et al.* 2020
	7	High Effective of 14-Day High-Dose PPI- Bismuth-Containing Quadruple Therapy with Probiotics Supplement for *Helicobacter Pylori* Eradication: A Double Blinded-Randomized Placebo-Controlled Study	Poonyam *et al.* 2019
	8	Effectiveness of 7-Day and 14-Day Moxifloxacin-Dexlansoprazole Based Triple Therapy and Probiotic Supplement for *Helicobacter Pylori* Eradication in Thai Patients with Non-Ulcer Dyspepsia: A Double-Blind Randomized Placebo-Controlled Study	Chotivitayatarakorn *et al.* 2017
	9	Improved *Helicobacter pylori* eradication rate of tailored triple therapy by adding *Lactobacillus delbrueckii* and *Streptococcus thermophilus* in northeast region of Thailand: A prospective randomized controlled clinical trial	Tongtawee *et al.* 2015
	10	Eradication of *Helicobacter pylori* infection with a new bismuth-based quadruple therapy in clinical practice	Pérez-Arellano *et al.* 2018
	11	Are probiotics useful in *Helicobacter pylori* eradication?	Homan & Orel 2015
	12	Effect of *Clostridium butyricum* on fecal flora in *Helicobacter pylori* eradication therapy	Shimbo *et al.* 2005
	13	Brief report: *Lactobacillus bulgaricus* GLB44 (Proviotic™) plus esomeprazole for *Helicobacter pylori* eradication: A pilot study	Opekun *et al.* 2018
	14	*Helicobacter pylori* eradication: A randomized prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics	Bortoli *et al.* 2007
	15	Evaluation of the potential inhibitory activity of a combination of *L. acidophilus*, *L. rhamnosus* and *L. sporogenes* on *Helicobacter pylori*: A randomized double-blind placebo-controlled clinical trial	Lee *et al.* 2016
	16	The effect of *Lactobacillus reuteri* supplementation in *Helicobacter pylori* infection: a placebo-controlled, single-blind study	Buckley *et al.* 2018
Duplicate	1	Effect of pretreatment with *Lactobacillus gasseri* OLL2716 on first line *Helicobacter pylori* eradication therapy	Deguchi *et al.* 2012
	2	Effect of *Lactobacillus casei* supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection	Tursi *et al.* 2004
	3	Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: A parallel group, triple blind, placebo-controlled study	Cremonini *et al.* 2002

Source: Own elaboration.

Annex 2. Exploration of risk of publication bias.

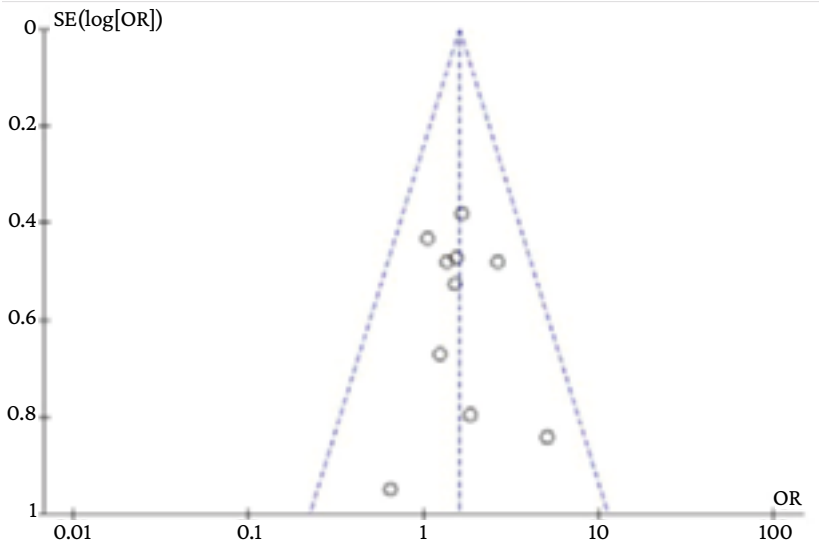

Figure 2.1. Assessment of biases in the included studies.
Source: Own elaboration.

Annex 3. Pooled measures of effect for other outcomes.

The figures below show the studies or subgroups from which the effect was obtained, the number of events, and the total number of participants for each group. Triple therapy or quadruple therapy plus probiotics were labeled as probiotics in the graphs and triple therapy plus placebo as placebo. The pooled measure was obtained using OR likelihood ratios and Mantel-Haenszel fixed effects models. In addition, the x^2 and I^2 values and the Z value of the statistical significance test for the pooled measure of effect are reported in the figures.

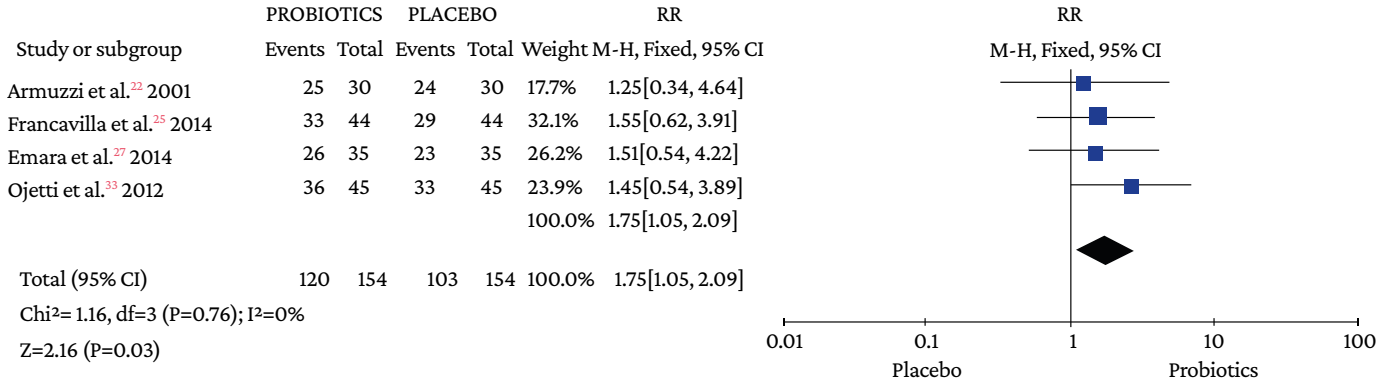

Figure 3.1. Eradication of *Helicobacter pylori* using *Lactobacillus reuteri*.
Source: Own elaboration.

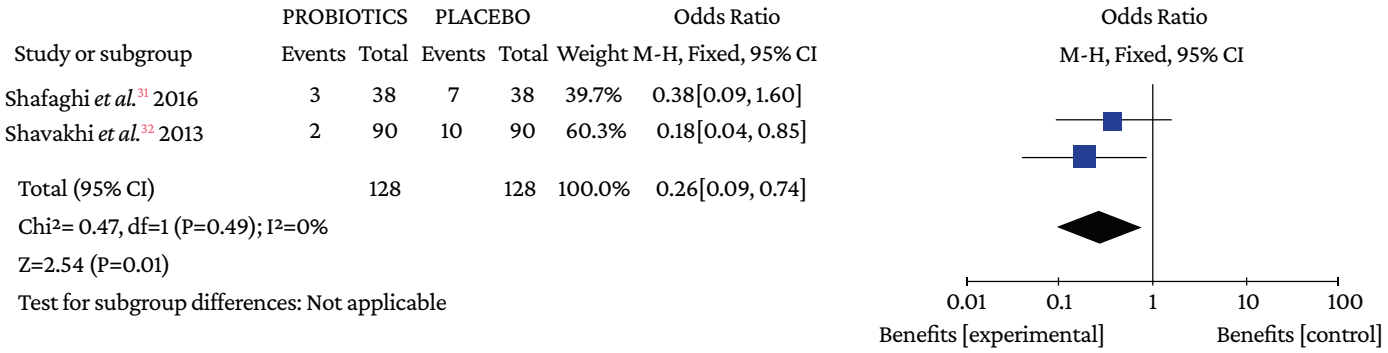

Figure 3.2. Any adverse event in quadruple therapy.
Source: Own elaboration.

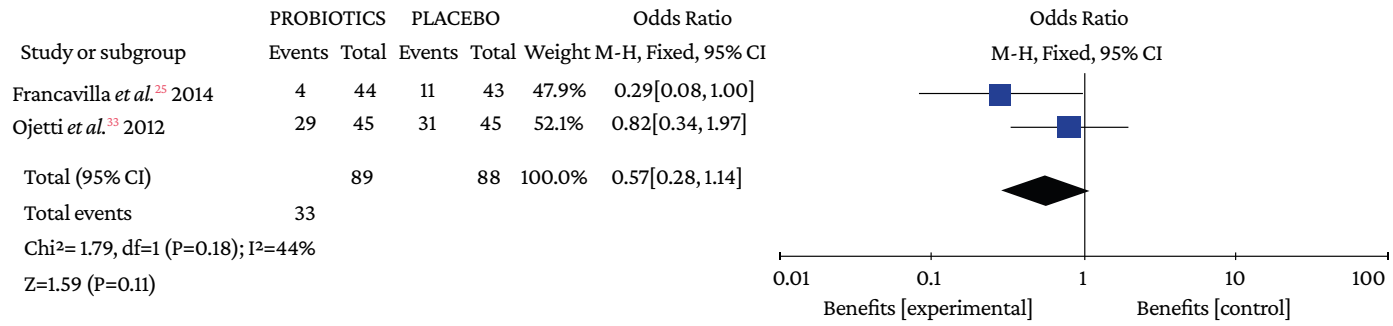

Figure 3.3. Abdominal pain in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	2	30	4	30	12.0%	0.46[0.08, 2.75]
Francavilla *et al.*[25] 2014	1	44	2	43	6.4%	0.48[0.04, 5.46]
Myllyluoma *et al.*[28] 2005	2	23	3	24	8.6%	0.67[0.10, 4.41]
Nista *et al.*[30] 2004	9	54	19	52	51.8%	0.35[0.14, 0.86]
Ojetti *et al.*[33] 2012	36	45	33	45	21.2%	1.45[0.54, 3.89]
Total (95% CI)		196		194	100.0%	0.63[0.36, 1.11]
Total events	50					
Chi²= 4.58, df=4 (P=0.33); I²=13%						
Z=1.61 (P=0.11)						

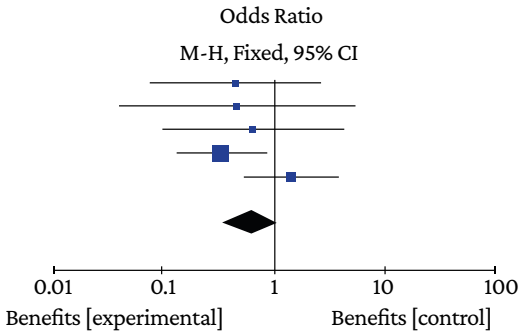

Figure 3.4. Anorexia in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	1	30	2	30	11.8%	0.48[0.04, 5.63]
Cindoruk *et al.*[24] 2007	2	62	2	62	11.8%	1.00[0.14, 7.33]
Myllyluoma *et al.*[28] 2005	0	23	1	24	8.8%	0.33[0.01, 8.61]
Nista *et al.*[30] 2004	4	54	8	54	45.3%	0.46[0.13, 1.63]
Ojetti *et al.*[33] 2012	4	45	4	45	22.3%	1.00[0.23, 4.27]
Total (95% CI)		214		215	100.0%	0.64[0.29, 1.38]
Total events	11		17			
Chi²= 1.02, df=4 (P=0.91); I²=0%						
Z=1.15 (P=0.25)						

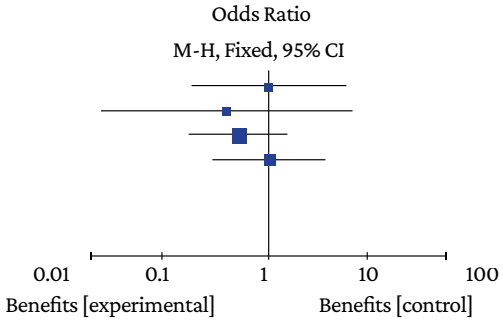

Figure 3.5. Skin rash in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	10	30	9	30	9.6%	1.17[0.39, 3.47]
Chitapanarux *et al.*[23] 2015	1	31	2	32	3.1%	0.50[0.04, 5.81]
Cindoruk *et al.*[24] 2007	8	62	20	62	28.0%	0.31[0.12, 0.78]
Francavilla *et al.*[25] 2014	3	44	9	43	13.6%	0.28[0.07, 1.10]
Myllyluoma *et al.*[28] 2005	4	23	7	24	9.1%	0.51[0.13, 2.06]
Nista *et al.*[30] 2004	24	54	34	52	30.9%	0.42[0.19, 5.12]
Ojetti *et al.*[33] 2012	5	45	4	45	5.7%	1.28[0.32, 5.12]
Total (95% CI)		289		288	100.0%	0.50[0.33, 0.77]
Total events	55		85			
Chi²= 6.01, df=6 (P=0.42); I²=0%						
Z=3.20 (P=0.001)						

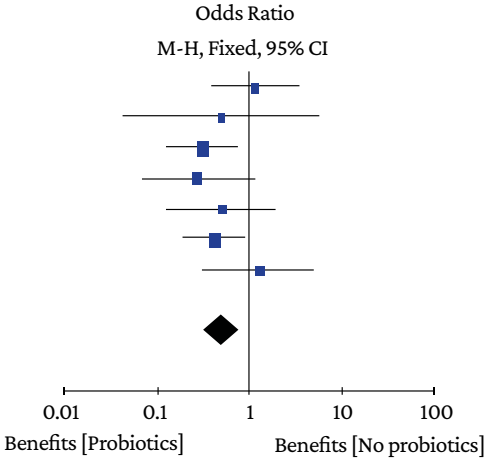

Figure 3.6. Pain in the epigastric region in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	3	30	11	30	14.8%	0.19[0.05, 0.78]
Chitapanarux *et al.*[23] 2015	4	31	6	32	7.7%	0.64[0.16, 2.54]
Cindoruk *et al.*[24] 2007	6	62	11	62	14.8%	0.50[0.17, 1.44]
Francavilla *et al.*[25] 2014	2	44	5	43	7,2%	0.36[0.07, 1.98]
Myllyluoma *et al.*[28] 2005	3	23	5	24	6.3%	0.57[0.12, 2.72]
Nista *et al.*[30] 2004	14	54	26	52	29.3%	0.35[0.15, 0.79]
Ojetti *et al.*[33] 2012	32	45	45	45	19.9%	0.03[0.00, 0.46]
Total (95% CI)		289		288	100.0%	0.32[0.20, 0.51]
Total events	64		109			
Chi²= 5.64, df=6 (P=0.46); I²=0%						
Z=4.80 (P=0.00001)						

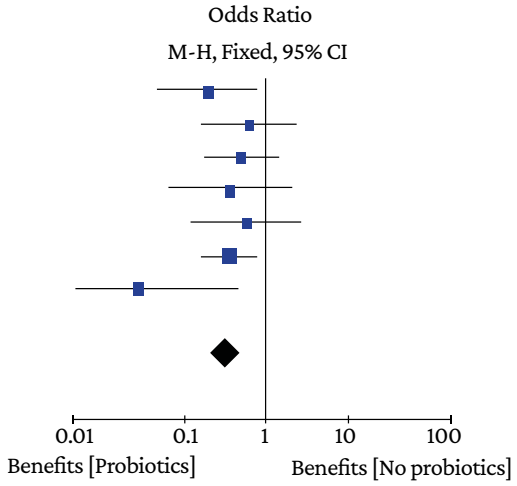

Figure 3.7. Nausea in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	1	30	8	30	8.9%	0.09[0.01, 0.82]
Chitapanarux *et al.*[23] 2015	1	31	8	32	8.8%	0.10[0.01, 0.86]
Cindoruk *et al.*[24] 2007	7	62	16	62	16.3%	0.37[0.14, 0.97]
Francavilla *et al.*[25] 2014	4	44	12	43	12.7%	0.26[0.08, 0.88]
Emara *et al.*[27] 2014	1	35	10	35	11.2%	0.07[0.01, 0.61]
Myllyluoma *et al.*[28] 2005	4	23	2	24	1.9%	2.32[0.38, 14.08]
Nista *et al.*[30] 2004	5	54	16	52	17.0%	0.23[0.08, 0.68]
Ojetti *et al.*[33] 2012	10	45	26	45	23.3%	0.21[0.08, 0.52]
Total (95% CI)		324		323	100.0%	0.25[0.16, 0.39]
Total events	33		98			
Chi²= 9.37, df=7 (P=0.23); I²=25%						
Z=6.18 (P=0.00001)						

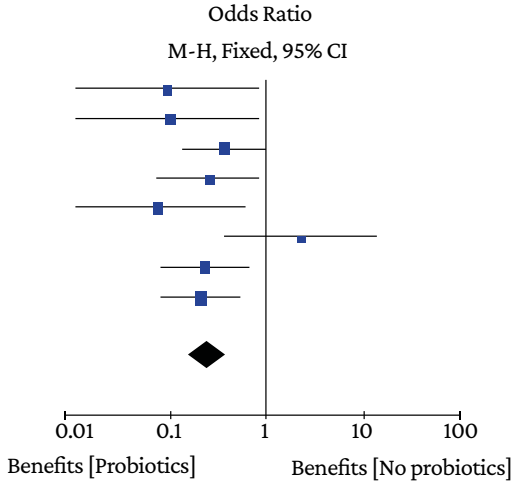

Figure 3.8. Diarrhea in triple therapy.
Source: Own elaboration.

Study or subgroup	PROBIOTICS Events	Total	PLACEBO Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Armuzzi *et al.*[22] 2001	7	30	15	30	19.5%	0.30[0.10, 0.92]
Chitapanarux *et al.*[23] 2015	4	31	5	32	7.2%	0.80[0.19, 3.31]
Cindoruk *et al.*[24] 2007	4	62	6	62	9.5%	0.64[0.17, 2.40]
Francavilla *et al.*[25] 2014	2	44	4	43	6.5%	0.46[0.08, 0.88]
Emara *et al.*[27] 2014	2	35	8	35	12.8%	0.20[0.04, 1.04]
Myllyluoma *et al.*[28] 2005	16	23	16	24	8.1%	1.14[0.33, 3.90]
Nista *et al.*[30] 2004	29	54	31	52	24.7%	0.79[0.36, 1.70]
Ojetti *et al.*[33] 2012	6	45	8	45	11.7%	0.71[0.23, 2.25]
Total (95% CI)		324		323	100.0%	0.60[0.40, 0.91]
Total events	70		93			
Chi²= 4.97, df=7 (P=0.66); I²=0%						
Z=2.40 (P=0.02)						

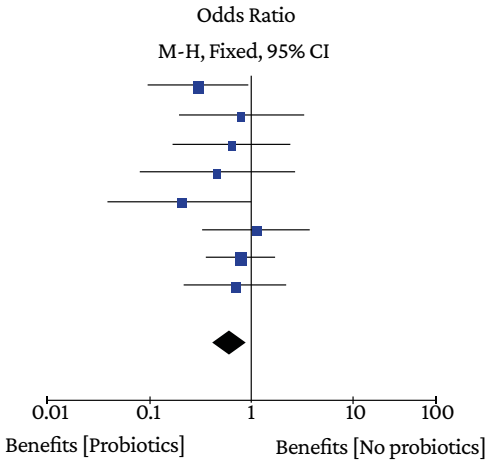

Figure 3.9. Taste disturbance in triple therapy.
Source: Own elaboration.

Annex 4. Assessment of the certainty of evidence.

Table 4.1. Assessment of triple therapy using GRADE: adjuvant probiotics vs. adjuvant placebo.

Certainty of evidence							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirect evidence	Inaccuracy	Other considerations	Probiotics	Placebo or no intervention	Relative (95%CI)	Absolute (95%CI)		
***H. pylori* eradication (follow-up: range 4 weeks to 8 weeks)**												
10	Randomized trials	Serious*	Not serious	Not serious	Not serious	None	328/413 (79.41%)	290/408 (71.07%)	OR 1.59 (1.15-2.20)	85 more per 1 000 (from 28 more to 133 more)	⊕⊕⊕◯ MODERATE	Critical
Adverse events (follow-up: range 1 week to 8 weeks)												
2	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	50/98 (51.02%)	64/95 (67.36%)	OR 0.50 (0.28-0.90)	166 less per 1 000 (from 307 less to 24 less)	⊕⊕⊕⊕ HIGH	Critical
Abdominal pain (follow-up: range 1 week to 8 weeks)												
2	Randomized trials	Very serious †	Not serious	Not serious	Very serious	None	33/89 (37.07%)	42/88 (47.72%)	OR 0.54 (0.20-1.45)	147 less per 1 000 (from 323 less to 92 more)	⊕◯◯◯ VERY LOW	Important
Hyporexia (follow-up: range 1 week to 2 weeks)												
5	Randomized trials	Serious †	Not serious	Not serious	Serious ‡	None	50/196 (25.51%)	61/194 (31.44%)	OR 0.63 (0.36-1.11)	90 less per 1 000 (from 173 less to 23 more)	⊕⊕◯◯ LOW	Important
Pain in the epigastric region (follow-up: range 1 week to 4 weeks)												
7	Randomized trials	Very serious †,**	Not serious	Not serious	Not serious	None	55/289 (19.03%)	85/288 (29.51%)	OR 0.55 (0.33-0.77)	108 less per 1 000 (from 174 less to 51 less)	⊕⊕◯◯ LOW	Critical
Nausea (follow-up: range 1 week to 4 weeks)												
7	Randomized trials	Very serious †,**	Not serious	Not serious	Not serious	Strong association	64/289 (22.14%)	109/288 (37.84%)	OR 0.32 (0.20-0.51)	215 less per 1 000 (from 270 less to 142 less)	⊕⊕⊕◯ MODERATE	Critical
Diarrhea (follow-up: range 1 week to 4 weeks)												
8	Randomized trials	Serious †,**	Not serious	Not serious	Not serious	Strong association	33/324 (10.18%)	98/323 (30.34%)	OR 0.25 (0.16-0.39)	205 less per 1 000 (from 238 less to 158 less)	⊕⊕⊕⊕ HIGH	Critical
Taste disturbance (follow-up: range 1 week to 4 weeks)												
8	Randomized trials	Serious †,**	Not serious	Not serious	Not serious	None	70/324 (21.60%)	93/323 (28.79%)	OR 0.60 (0.40-0.91)	93 less per 1 000 (from 149 less to 19 less)	⊕⊕⊕◯ MODERATE	Important

Certainty of evidence							No. of patients		Effect		Certainty	Importance
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirect evidence**	**Inaccuracy**	**Other considerations**	**Probiotics**	**Placebo or no intervention**	**Relative (95%CI)**	**Absolute (95%CI)**		
Skin rash (follow-up: 1 week on average)												
5	Randomized trials	Serious †,**	Not serious	Not serious	Very serious ‡	None	11/214 (5.14%)	17/215 (7.90%)	OR 0.64 (0.29-1.38)	27 less per 1 000 (from 55 less to 27 more)	⊕◯◯◯ VERY LOW	Important

* Most studies raised concerns about selection bias and in 2 of 10 studies there were concerns about underreporting of results.
† Study at high risk of bias in 4 key domains.
‡ Estimates with +confidence index crossing the no effect line, few outcomes, and few participants.
** Most studies have a risk of uncertain selection bias.
Source: Own elaboration.

Table 4.2. GRADE assessment for quadruple therapy: adjuvant probiotics vs. adjuvant placebo.

Certainty of evidence							No. of patients		Effect		Certainty	Importance
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirect evidence**	**Inaccuracy**	**Other considerations**	**Probiotics**	**Placebo**	**Relative (95%CI)**	**Absolute (95%CI)**		
***H. pylori* eradication (follow-up: range 2 weeks to 4 weeks)**												
3	Randomized trials	Serious *	Not serious	Not serious	Serious †	Strong association	171/195 (87.69%)	164/198 (82.82%)	OR 2.67 (0.44-16.14)	100 more per 1 000 (from 149 less to 159 more)	⊕⊕⊕◯ MODERATE	Critical
Diarrhea (follow-up: 1 month on average)												
2	Randomized trials	Serious *	Not serious	Not serious	Not serious	Strong association	5/128 (3.90%)	17/128 (13.28%)	OR 0.26 (0.09-0.74)	95 less per 1 000 (from 119 less to 31 less)	⊕⊕⊕⊕ HIGH	Critical

* Risk of unclear bias in the allocation and random sequence generation domains.
† Inaccuracy by estimator and CI cross the no-difference line, few participants.
Source: Own elaboration.