Full search strings strategy utilized for each database for the selection of randomized controlled trials

Pubmed: ("Helicobacter" OR "Helicobacter Pylori" OR "H pylori" OR "Hp" OR "Campylobacter Pylori") AND ("Lactobacillus reuteri" OR "L. reuteri" OR "Lactobacillus reuteri ATCC 55730" OR "L. reuteri ATCC 55730" OR "Lactobacillus reuteri DSM 17938" OR "L. reuteri DSM 17938").

Embase and the Cochrane Library: "Helicobacter" OR "Helicobacter Pylori" OR "H pylori" OR "Hp" OR "Campylobacter Pylori" AND "Lactobacillus reuteri" OR "L. reuteri" OR "Lactobacillus reuteri ATCC 55730" OR "L. reuteri ATCC 55730" OR "Lactobacillus reuteri DSM 17938" OR "L. reuteri DSM 17938".

PRISMA 2020 checklist compliance document

16b. Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.

Several studies that initially appeared eligible were ultimately excluded after full-text evaluation due to failure to meet one or more inclusion criteria. For instance:

Zullo et al. (2007)[1] and Zou et al. (2009)[2] were excluded because they used mixed or undefined probiotic formulations that did not include *L. reuteri* DSM 17938 specifically.

Lü et al. (2016)[3] and Li et al. (2024)[4] were excluded because their meta-analyses included heterogeneous strains of *Lactobacillus*, such as DSM 17648 and ATCC PTA 6475 without clearly isolating data specific to DSM 17938.

Glintborg et al. (2006)[5], was excluded as the control group did not have H. pylori infection.

Efrati et al. (2012)[6] was excluded because it is not double-blind (no control group), does not report eradication numbers per group, and does not specify the duration of probiotic therapy in relation to antibiotic treatment.

Imase et al. (2007)[7] was excluded because it reports only combined eradication rates for both groups and focuses on UBT reduction rather than actual eradication.

Dore et al (2019)[8] was excluded because it used only a probiotic combined with a proton pump inhibitor (PPI), without the use of antibiotics.

Review articles, animal studies, and conference abstracts were systematically excluded to ensure methodological rigor, as they did not provide primary clinical outcome data from randomized controlled trials.

Studies lacking accessible full texts or outcome data were also excluded, despite appearing relevant during title and abstract screening.

These exclusions were essential to preserve the internal validity of the meta-analysis, which focused exclusively on RCTs evaluating the strain *L. reuteri* DSM 17938 (alone or in combination with ATCC PTA 6475) as an adjunct to standard *Helicobacter pylori* eradication therapy.

References

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2. Zou J, Dong J, Yu X. Meta-analysis: Lactobacillus containing quadruple therapy versus standard triple first-line therapy for Helicobacter pylori eradication. Helicobacter. 2009;14(5):97-107. doi:10.1111/j.1523-5378.2009.00716.x
3. Lü M, Yu S, Deng J, et al. Efficacy of Probiotic Supplementation Therapy for Helicobacter pylori Eradication: A Meta-Analysis of Randomized Controlled Trials. PLoS One. 2016;11(10):e0163743. Published 2016 Oct 10. doi:10.1371/journal.pone.0163743
4. Li M, Wang X, Dong X, et al. Lactobacillus reuteri compared with placebo as an adjuvant in Helicobacter pylori eradication therapy: a meta-analysis of randomized controlled trials. Therap Adv Gastroenterol. 2024;17:17562848241258021
5. Glintborg, B., Dawids, S., Hasselby, J. P., et al. "LONG-TERM ADMINISTRATION OF LACTOBACILLUS REUTERI (ATCC55730) HAS NOINFLUENCE ON GASTRIC MUCOSAL INFLAMMATION AND COLONIZATION OF HELICOBACTER PYLORI IN HUMANS. A PILOT STUDY." INTERNATIONAL JOURNAL OF PROBIOTICS AND PREBIOTICS 1.3/4 (2006): 219.

6. Efrati C, Nicolini G, Cannaviello C, O'Sed NP, Valabrega S. Helicobacter pylori eradication: sequential therapy and Lactobacillus reuteri supplementation. World J Gastroenterol. 2012;18(43):6250-6254.
7. Imase K, Tanaka A, Tokunaga K, Sugano H, Ishida H, Takahashi S. Lactobacillus reuteri tablets suppress Helicobacter pylori infection--a double-blind randomised placebo-controlled cross-over clinical study. Kansenshogaku Zasshi. 2007;81(4):387-393.
8. Dore MP, Bibbò S, Pes GM, et al. Role of Probiotics in Helicobacter pylori Eradication: Lessons from a Study of Lactobacillus reuteri Strains DSM 17938 and ATCC PTA 6475 (Gastrus®) and a Proton-Pump Inhibitor. Can J Infect Dis Med Microbiol. 2019;2019:3409820.

20d. Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.

Sensitivity Analysis Results

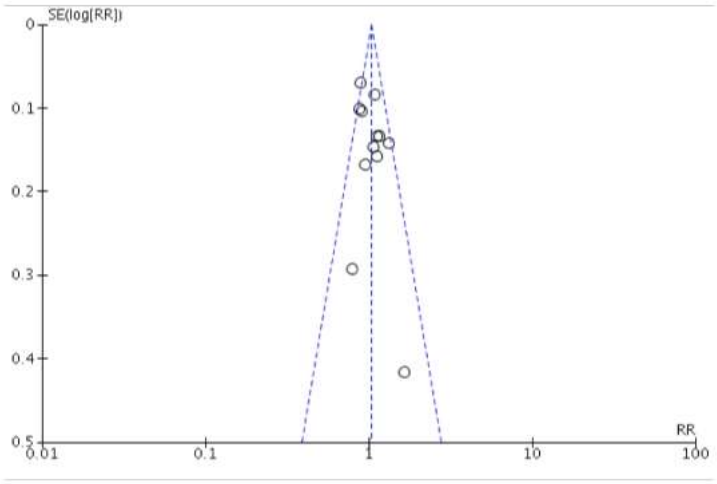
Sensitivity analyses were conducted through **pre-specified subgroup evaluations**, confirming the robustness and consistency of the synthesized results:

- **By population type**: Among adults (10 studies), the pooled RR for *H. pylori* eradication was 1.01 (95% CI: 0.91–1.11; $I^2 = 40.1\%$); among children (2 studies), RR was 1.11 (95% CI: 0.92–1.35; $I^2 = 0.0\%$).
- **By antibiotic regimen**:
  - Bismuth-based quadruple therapy: RR 0.94 (95% CI: 0.86–1.03; $I^2 = 9.8\%$)
  - Standard triple therapy: RR 1.10 (95% CI: 0.91–1.33; $I^2 = 0.0\%$)
  - Sequential therapy: RR 1.12 (95% CI: 0.93–1.35; $I^2 = 0.0\%$)
  - Levofloxacin-based therapy: RR 1.33 (95% CI: 1.01–1.76; single study)
- **By treatment duration**:
  - 10-day regimens: RR 0.94 (95% CI: 0.85–1.04; $I^2 = 16.8\%$)
  - 14-day regimens: RR 1.11 (95% CI: 0.98–1.26; $I^2 = 0.0\%$)
  - 7-day regimens: RR 1.06 (95% CI: 0.79–1.43; $I^2 = 48.0\%$)

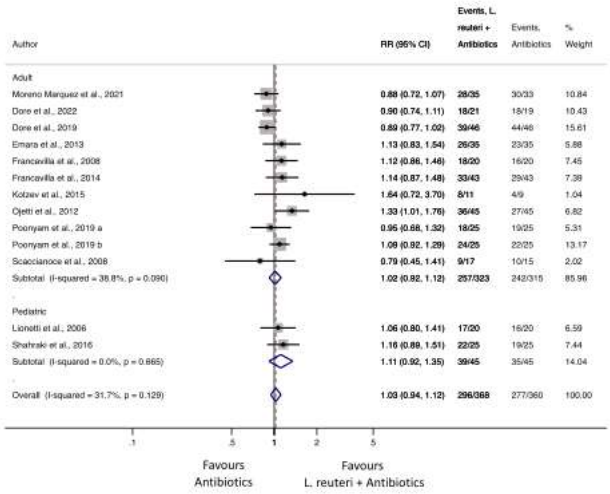
All subgroup analyses showed **non-significant differences**, except for the levofloxacin subgroup (based on a single study), reinforcing the conclusion that *L. reuteri* DSM 17938 does not significantly enhance eradication efficacy.

Regarding adverse events, subgroup sensitivity analyses confirmed a **consistent protective effect** of *L. reuteri* across populations and regimens for nausea (RR 0.50), diarrhoea (RR 0.45), and abdominal pain (RR 0.59), all with negligible heterogeneity (I^2 = 0–28%).

No formal leave-one-out analysis was performed, but the consistent findings across subgroups and the use of a random-effects model provide strong evidence for the robustness of the synthesized results.

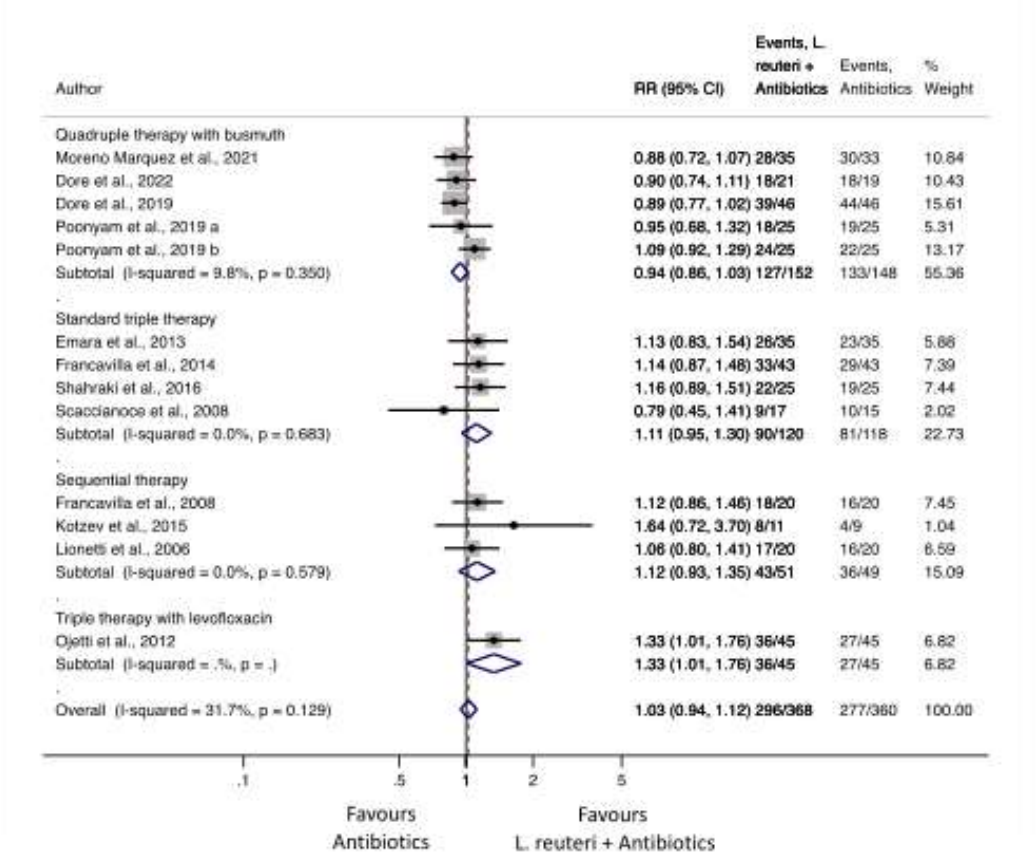


Supplementary Figure 1 Funnel plot assessing publication bias in the meta-analysis of *Helicobacter pylori* eradication rates with or without *Limosilactobacillus reuteri* supplementation. Each circle represents an individual study plotted by the standard error (SE) of the log risk ratio (RR) against the RR. The symmetrical distribution of studies around the central effect estimate and within the pseudo 95%confidence limits suggests a low risk of publication bias.

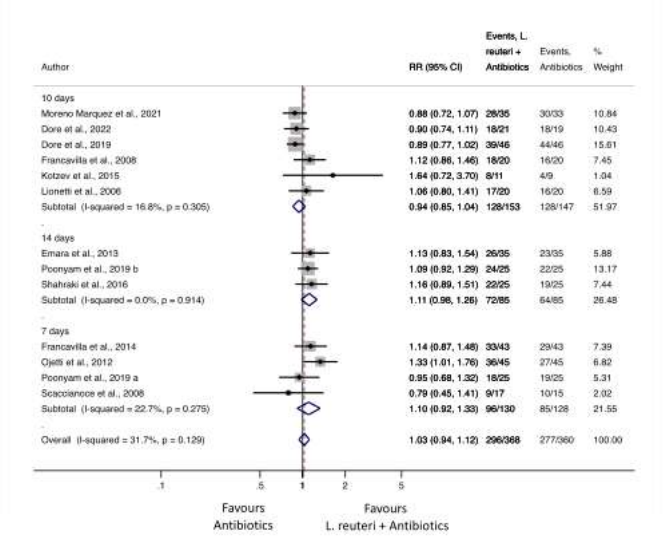


Supplementary Figure 2 Subgroup analysis of the effect of *Limosilactobacillus reuteri* supplementation on *Helicobacter pylori* eradication rates, stratified by population type (adult vs. pediatric). The pooled risk ratio (RR) for adult populations was 1.02 (95% CI: 0.92–1.12; $I^2 = 38.8\%$, $p = 0.090$), while in pediatric populations the RR was 1.11 (95% CI: 0.92–1.35;

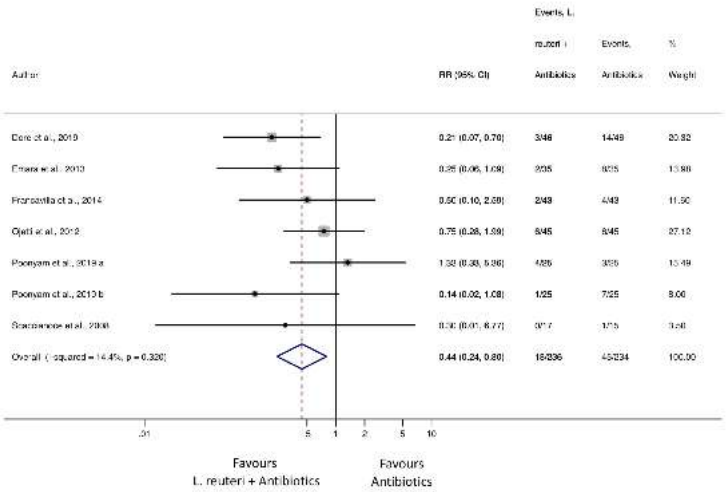
I^2 = 0.0%, p = 0.665). The overall RR across all studies was 1.03 (95% CI: 0.94–1.12; I^2 = 31.7%, p = 0.129), indicating no statistically significant difference in eradication efficacy between *L. reuteri* + antibiotics versus antibiotics alone across populations.



Supplementary Figure 3 Subgroup analysis of the effect of *Limosilactobacillus reuteri* supplementation on *Helicobacter pylori* eradication rates, stratified by type of antibiotic regimen. The pooled risk ratio (RR) for quadruple therapy with bismuth was 0.94 (95% CI: 0.86–1.03; I^2 = 9.8%, p = 0.350), for standard triple therapy 1.11 (95% CI: 0.95–1.30; I^2 = 0.0%, p = 0.683), for sequential therapy 1.12 (95% CI: 0.93–1.35; I^2 = 0.0%, p = 0.579), and for the single study evaluating levofloxacin-based triple therapy the RR was 1.33 (95% CI: 1.01–1.76). No statistically significant differences in eradication rates were found across subgroups, except for the levofloxacin-based regimen, which showed a marginally significant benefit in favor of *L. reuteri* supplementation.

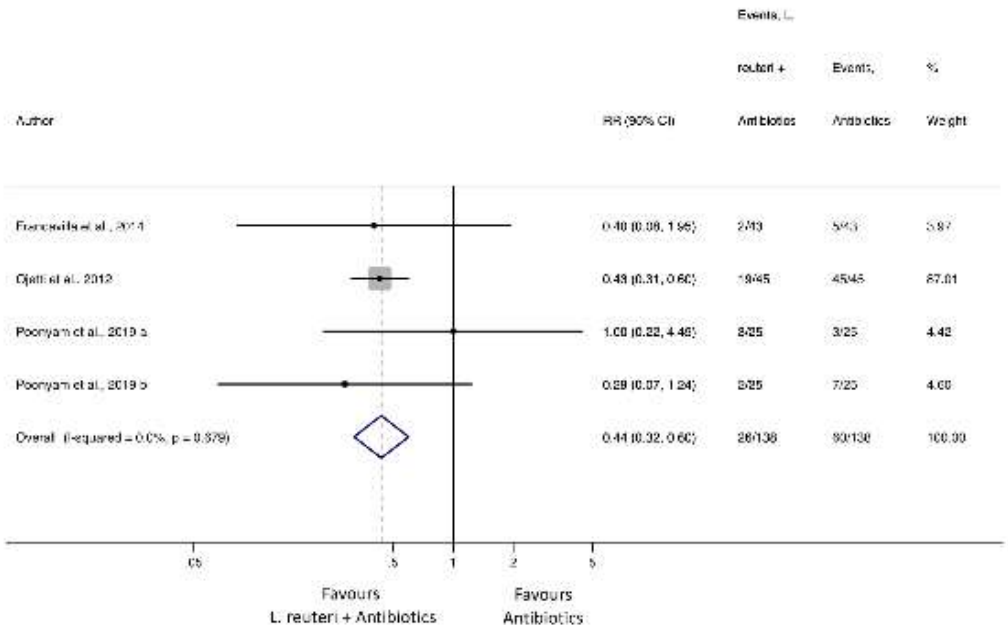


Supplementary Figure 4 Subgroup analysis of the effect of *Limosilactobacillus reuteri* supplementation on *Helicobacter pylori* eradication rates, stratified by duration of antibiotic therapy. In studies with 10-day regimens, the pooled risk ratio (RR) was 0.94 (95% CI: 0.85–1.04; I^2 = 16.8%, p = 0.305), while for 14-day regimens the RR was 1.11 (95% CI: 0.98–1.26; I^2 = 0.0%, p = 0.914). Studies with 7-day regimens yielded a pooled RR of 1.10 (95% CI: 0.92–1.33; I^2 = 22.7%, p = 0.275). No statistically significant differences in eradication outcomes were observed among these subgroups.

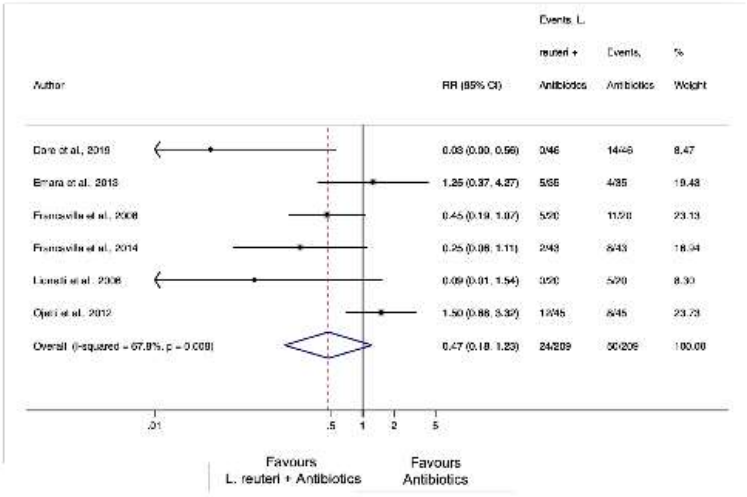


Supplementary Figure 5 Forest plot showing the effect of *Limosilactobacillus reuteri* supplementation on the incidence of dysgeusia during *Helicobacter pylori* eradication

therapy. The pooled analysis includes data from seven randomized controlled trials comparing *L. reuteri* plus antibiotics versus antibiotics alone. The overall risk ratio (RR) was 0.44 (95% CI: 0.24–0.80), indicating a statistically significant 56% relative risk reduction in dysgeusia associated with *L. reuteri* administration. Heterogeneity was low (I^2 = 14.4%, p = 0.320), reflecting consistent results across studies.



Supplementary Figure 6 Forest plot illustrating the effect of *Limosilactobacillus reuteri* supplementation on the incidence of nausea during *Helicobacter pylori* eradication therapy. The analysis includes four randomized controlled trials comparing *L. reuteri* plus antibiotics to antibiotics alone. The pooled risk ratio (RR) was 0.44 (95% CI: 0.32–0.60), indicating a statistically significant 56% reduction in nausea risk with *L. reuteri* administration. Heterogeneity across studies was negligible (I^2 = 0.0%, p = 0.679), supporting the consistency of this effect.



Supplementary Figure 7 Forest plot of randomized controlled trials evaluating the incidence of bloating in patients undergoing *Helicobacter pylori* eradication therapy with or without *Limosilactobacillus reuteri* supplementation. The analysis includes six studies. The pooled risk ratio (RR) for bloating was 0.47 (95% CI: 0.18–1.23), suggesting a trend toward reduced bloating with *L. reuteri* supplementation, although the result did not reach statistical significance. Heterogeneity across studies was moderate to high (I^2 = 67.8%; p = 0.008), indicating variability in effect estimates among trials.

Supplementary Table 1 Summary of subgroup analyses evaluating the effect of *Limosilactobacillus reuteri* supplementation on *Helicobacter pylori* eradication rates, stratified by population (adults vs. children), antibiotic regimen, and treatment duration

Subgroup	No. of Studies	Pooled RR (95% CI)	Heterogeneity (I^2, p)	P
Adults	10	1.01 (0.91–1.11)	40.1%, p = 0.090	No
Children	2	1.11 (0.92–1.35)	0.0%, p = 0.665	No
Quadruple therapy with bismuth	5	0.94 (0.86–1.03)	9.8%, p = 0.350	No
Standard triple therapy	3	1.10 (0.91–1.33)	0.0%, p = 0.474	No
Sequential therapy	3	1.12 (0.93–1.35)	0.0%, p = 0.579	No
Triple therapy with levofloxacin	1	1.33 (1.01–1.76)	—	—
10-day regimens	6	0.94 (0.85–1.04)	16.8%, p = 0.305	No
14-day regimens	3	1.11 (0.98–1.26)	0.0%, p = 0.914	No
7-day regimens	3	1.06 (0.79–1.43)	48.0%, p = 0.146	No

Pooled risk ratios (RR) with 95% confidence intervals (CI), heterogeneity statistics (I^2 and p-values), and statistical significance are reported for each subgroup. P-values refer to the statistical significance of the effect estimate (RR) within each subgroup. No test for subgroup interaction was performed.

Supplementary Table 2 Summary of subgroup analyses evaluating the effect of Limosilactobacillus reuteri supplementation during antibiotic therapy on gastrointestinal symptoms and related antibiotic-associated adverse events

Subgroup	Studies	Pooled RR (95% CI)	Heterogeneity (I^2, p)	P
Dysgeusia *(antibiotic type)*	3	0.89 (0.64–1.24)	41.5%,	p = 0.46
Dysgeusia *(duration)*	3	0.83 (0.52–1.34)	56.5%	p = 0.47
Nausea *(antibiotic type)*	2	0.50 (0.30–0.84)	0.0%,	p = 0.007

Vomiting *(population)*	2	0.92 (0.52–1.64)	0.0%,	p = 0.775
Vomiting *(antibiotic type)*	3	1.17 (0.75–1.83)	28.1%	p = 0.474
Vomiting *(duration)*	3	0.77 (0.33–1.79)	0.0%	p = 0.537
Diarrhea *(antibiotic type)*	3	0.45 (0.24–0.83)	0.0%	p = 0.011
Diarrhea *(duration)*	3	0.48 (0.21–1.09)	27.7%	p = 0.077
Abdominal pain *(population)*	2	0.59 (0.35–0.98)	0.0%	p = 0.044
Abdominal pain *(antibiotic type)*	3	0.74 (0.41–1.33)	54.5%	p = 0.292
Abdominal pain *(duration)*	3	0.95 (0.45–2.01)	63.6%	p = 0.896
Meteorism *(population)*	2	0.43 (0.15–1.23)	18.4%	p = 0.115
Meteorism (antibiotic type)	2	0.60 (0.33–1.08)	0.0%	p = 0.083
Meteorism *(duration)*	2	0.33 (0.12–0.92)	0.0%	p = 0.033

For each subgroup analysis (e.g., by antibiotic type or treatment duration), data are reported separately. "Antibiotic type" includes subgroups such as clarithromycin-based (n=2) and levofloxacin-based (n=1) regimens; "duration" includes studies with 7-day (n=1), 10-day (n=1), and 14-day (n=1) treatment arms. The RR values reflect pooled comparisons within each subgroup. Each subgroup analysis is independent and should not be directly compared to others. Results are stratified by population type, antibiotic regimen, and treatment duration. Pooled risk ratios (RRs) with 95%

confidence intervals (CIs) are presented along with heterogeneity indices (I^2 and p-values). Statistical significance is indicated in the final column.

Supplementary Table 3 Subgroup comparison of single- *versus* multi-strain regimens in per-protocol participants

Outcome	Probiotic Regimen	N° of studies	Events, L. reuteri + Antibiotics	RR (95% CI)	OR (95% CI)	*p*-value
Eradication	DSM 17938	5	102/127	1.00	0.99	1.000
Eradication	DSM 17938 + PTA 6475	8	194/241	(0.90–1.11)	(0.58–1.70)	
Diarrhea	DSM 17938	2	10/62	3.32	3.77	0.023
Diarrhea	DSM 17938 + PTA 6475	3	5/103	(1.19–9.27)	(1.22–11.61)	
Dysgeusia	DSM 17938	2	6/62	1.40	1.45	0.577
Dysgeusia	DSM 17938 + PTA 6475	5	12/174	(0.55–3.58)	(0.52–4.04)	
Nausea	DSM 17938	1	19/45	5.61	8.98	<0.001
Nausea	DSM 17938 + PTA 6475	3	7/93	(2.55–12.36)	(3.40–23.71)	
Bloating	DSM 17938	3	17/85	3.54	4.18	0.002
Bloating	DSM 17938 + PTA 6475	3	7/124	(1.54–8.17)	(1.65–10.59)	

For each outcome, event counts (numerator/denominator) are shown for the probiotic (L. reuteri + antibiotics). Risk ratios (RR) and odds ratios (OR) with 95% confidence intervals (CI), and two-sided p-values (Fisher's exact test) compare the single-strain (DSM 17938) versus multi-strain (DSM 17938 + PTA 6475) regimens using the probiotic arms only.

Supplementary Table 4 Summary of findings: comparison of antibiotics plus *Limosilactobacillus reuteri* vs. antibiotics, assessed according to the GRADE approach

Outcome	No. of participants (no. of studies)	Relative effect, RR (95% CI)	Certainty of the evidence (GRADE)	Reasons for downgrading certainty in evidence
H. pylori eradication	728 (13)	1.03 (0.94 to 1.12)	Moderate ●●●○	Downgrade 1 level for inconsistency*
Reduction in adverse events				
Dysgeusia	443 (7)	**0.44 (0.24 to 0.80)**	Low ●●○○	Downgrade 2 levels for quality of included studies and inconsistency§
Nausea	276 (4)	**0.44 (0.32 to 0.60)**	Moderate ●●●○	Downgrade 1 level for inconsistency§
Vomiting	464 (7)	0.94 (0.60 to 1.47)	Moderate ●●●○	Downgrade 1 level for inconsistency*
Epigastric pain	216 (3)	0.51 (0.22 to 1.19)	Very low ●○○○	Downgrade 3 levels for quality of included studies, imprecision and inconsistency*
Diarrhea	378 (6)	**0.34 (0.21 to 0.55)**	Moderate ●●●○	Downgrade 1 level for inconsistency§
Constipation	176 (2)	0.69 (0.32 to 1.52)	Very low ●○○○	Downgrade 3 levels for quality of included studies, imprecision and inconsistency§
Abdominal pain	450 (7)	0.73 (0.41 to 1.33)	Low ●●○○	Downgrade 2 levels for quality of included studies and inconsistency*
Meteorism	418 (6)	0.47 (0.18 to 1.23)	Low ●●○○	Downgrade 2 levels for imprecision and inconsistency*

Asthenia	192 (3)	0.58 (0.18 to 1.85)	Low ●●○○	Downgrade 2 levels for quality of included study and imprecision
Vertigo	100 (2)	1.00 (0.26 to 3.78)	Low ●●○○	Downgrade 2 levels for imprecision
Appetite loss	86 (1)	0.50 (0.05 to 5.31)	Low ●●○○	Downgrade 2 levels for imprecision

RR, relative risk; CI, confidence interval

Significant results are reported in bold.

* Heterogeneity among populations enrolled (pediatric and adults) and antibiotic therapies used in included studies.

§ Heterogeneity among antibiotic therapies used in included studies.