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Probiotics for the Prevention of Antibiotic-associated Diarrhea in Adults

A Meta-Analysis of Randomized Placebo-Controlled Trials

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Objective: This meta-analysis aims to combine the latest research evidence to assess the effect of probiotics on preventing antibiotic-associated diarrhea (AAD) in adults.

Methods: PubMed, Cochrane Library, EMBASE, and Web of Science were searched for randomized placebo-controlled trials on probiotics preventing AAD. A random or fixed effect model was used to combine the incidence of AAD (primary outcome) and the adverse event rates. The authors performed subgroup analyses to explore the effects of different participants population, probiotics species, and dosage.

Results: Thirty-six studies were included with 9312 participants. Probiotics reduced the incidence of AAD by 38% (pooled relative risk, 0.62; 95% confidence interval, 0.51-0.74). The protective effect of probiotics was still significant when grouped by reasons for antibiotics treatment, probiotic duration, probiotic dosage, and time from antibiotic to probiotic. However, there were no statistically significant increased adverse events in the probiotics group (relative risk, 1.00; 95% confidence interval, 0.87-1.14).

Conclusions: This updated meta-analysis suggested that using probiotics as early as possible during antibiotic therapy has a positive and safe effect on preventing AAD in adults. Further studies should focus on the optimal dosage and duration of probiotics to develop a specific recommendation.

Key Words: probiotics, prevention, antibiotic-associated diarrhea, diarrhea, adults

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Antibiotic-associated diarrhea (AAD) is defined as diarrhea developing from the beginning of antibiotic treatment to 6 to 8 weeks after discontinuation, which may contribute to antimicrobial prescription noncompliance and the overconsumption of second-line antibiotics.[1] The prevalence of AAD varies between 5% and 39% in adults. It largely depends on the antibacterial spectrum and pharmacokinetic characteristics including the absorption rate of oral administration and enterohepatic circulation of parenteral administration.[2] The pathogenesis of AAD includes the following 2 aspects: (1) the direct effect of antibacterial agents on the intestinal mucosa; (2) the interference of antibacterial agents on the intestinal flora ecosystem, which leads to normal metabolic dysfunction and overgrowth of pathogens (especially *Clostridioides difficile*).[3]

As a live microorganism, probiotic with adequate amounts can bring health benefits to the host.[4] The mechanisms by which probiotics work on AAD may associate with the following: (1) altering the gut microbiota composition and metabolism; (2) modulating the solute secretion and absorption; and (3) improving the intestinal barrier function and intestinal immune responses.[5] Although several randomized controlled trials (RCTs) and meta-analyses have shown its efficacy in preventing AAD, there are currently no clear clinical practice guidelines for probiotics use in preventing AAD.[6] A review comparing the effectiveness of multiple probiotics suggested that positive or negative generalization about probiotics was inadequate. Strain specificity, the designated patient population, and various treatment conditions would change the effect of probiotics.[7] Therefore, our meta-analysis aims to combine the latest research evidence and compare the effects of probiotic

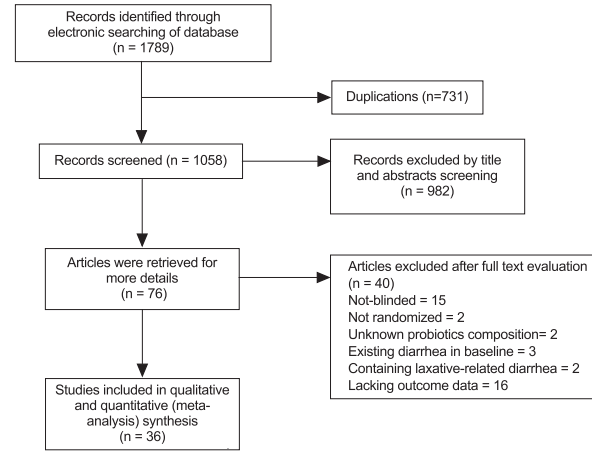

FIGURE 1. Selection process of meta-analysis.

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TABLE 1. Characteristics of Enrolled Studies

References	Risk of Bias (Based on Cochrane Handbook)	Setting	Sample Size (Treatment Group; Placebo Group)	Mean Age/ Range (Treatment Group; Placebo Group)	Diarrhea Definition	Antibiotic (s)	Time From Antibiotic to Probiotic, d	Probiotic Species	Dosage Per Day	Probiotic Duration (d)	Follow-up Period (From the Cessation of Antibiotics Treatment)
Armuzzi et al[18]	Low	Adults, asymptomatic	30/30	40	NR	*H. pylori* eradication	0	*Lactobacillus GG*	1.2×10^{10} CFU	14 d, AC†+7	3 wk
Thomas et al[19]	Low	Adults, in-patient	133/134	57.2/54.4	Other definition	Various	1	*Lactobacillus GG*	1×10^{10} CFU	14 d	1 wk
Cremonini et al[20]	Low	Adults, asymptomatic	63/20	18-61	NR	*H. pylori* eradication	0	*Lactobacillus GG, Saccharomyces boulardii,* or the combination of *L. acidophilus* and *Bifidobacterium lactis*	6×10^{9}, 5×10^{9}, or 5×10^{9} CFU	14 d, AC+7	3 wk
Nista et al[21]	Unclear	Adults, asymptomatic	54/52	46.0/43.0	NR	*H. pylori* eradication	0	*Bacillus clausii*	6×10^{9} CFU	14 d, AC+7	3 wk
Can et al[22]	Unclear	Adults, in-patient	73/78	25-50	NR	Various	2	*S. boulardii*	1×10^{10} CFU	Various, AC	4 wk
Beausoleil et al[23]	High	Adults, in-patient	44/45	68.8/72.9	WHO*	Various	2	A combination of *L. acidophilus* and *L. casei*	2.5×10^{10} CFU for the first 2 days, 5×10^{10v}CFU for the remaining days	Various, AC	3 wk
Cindoruk et al[24]	Unclear	adults	62/62	45.82/47.56	NR	*H. pylori* eradication	0	*S. boulardii*	1000 mg	14 d, AC	6 wk
Hickson et al[25]	Unclear	Adults, in-patient	57/56	73.7/73.9	Other definition	Various	2	A combination of *L. casei, S. thermophilus* and *L. bulgaricus*	1.94×10^{10}, 1.94×10^{10}, and 1.94×10^{9} CFU, respectively	Various, AC +7	4 wk
Bravo et al[26]	High	Adults, out-patient	41/45	49.78/50.98	WHO*	Amoxicillin	1	*S. boulardii*	1×10^{10} CFU	12 d, AC+ at least 2 d	At least 11 d
Koning et al[27]	Unclear	Adults, healthy volunteers	19/19	25.5/28.2	Other definition	Amoxycillin	0	A combination of *B. bifidum, B. lactis, B. Longum, E. faecium, L. acidophilus, L. paracasei, L. plantarum, L. rhamnosus L. salivarius*	1×10^{10} CFU	14 d, AC+7	8 wk

Wenus et al[28]	Unclear	Adults, in-patient	34/29	58.8/56.2	Adjusted WHO†	Various	3	A combination of *Lactobacillus GGL. acidophilus* and *Bifidobacterium*	2.50×10^{10}, 2.50×10^{9}, and 2.50×10^{10} CFU, respectively	14 d	0
Gao et al[29]	Unclear	Adults, in-patient	171/84	60/60	WHO*	One of penicillin, cephalo-sporin, or clindamycin	1.5	A combination of *L. acidophilus* and *L. casei*	5×10^{10} or 1×10^{11} CFU	Various, AC +5	26 d
Lonnermark et al[30]	Unclear	Adults, in-patient, and out-patient	80/83	47/43	Adjusted WHO†	Various	2	*L. plantarum*	1×10^{10} CFU	Various, AC +7	2 wk
Song et al[31]	High	Adults, in-patient	103/111	61/60	Adjusted WHO†	Various	2	A combination of *L. rhamnosus* and *L. acidophilus*	4×10^{9} CFU	14 d	0
Bekar et al[32]	Unclear	Adults	46/36	46/43	NR	*H. pylori* eradication	0	A combination of *Lactobacilli, lactic streptococci,* yeasts, and acetic acid bacteria	500 mL	14 d, AC	0
Cimperman et al[33]	High	Adults, in-patient	13/10	42.8/63.6	Adjusted WHO†	Various	4	*L. reuteri*	2×10^{8} CFU	28 d	2 wk
Manfredi et al[34]	Low	Adults	73/76	46.4/50.6	NR	*H. pylori* eradication	0	A combination of *L. acidophilus, L. bulgaricus, B. bifidum,* and *Streptococcus thermophilus*	2×10^{9}, 2×10^{9}, 1×10^{9}, and 2×10^{9} CFU, respectively	10 d, AC	0
Pozzoni et al[35]	Low	Adults, in-patient	106/98	79.9/78.5	Other definition	Various	2	*S. boulardii*	1×10^{10} CFU	Various, AC +7	12 wk
Allen et al[36]	Low	Adults, in-patient	1470/1471	77.2/77.0	WHO*	Various	7	A combination of *L. acidophilus, B. bifidum* and *B. lactis*	6×10^{10} CFU	21 d	5 wk
Chatterjee et al[37]	Low	Adults, out-patient	176/167	18-70	Adjusted WHO†	One of cefadroxil or amoxycillin	0	A combination of *L. acidophilus* and *Bifidobacterium*	4×10^{9} CFU	14 d, AC+7	1 wk
Padilla et al[38]	Unclear	Adults	29/30	56.6	NR	*H. pylori* eradication	0	*L. rhamnosus*	1.2×10^{10} CFU	7 d, AC	0

TABLE 1. (*continued*)

References	Risk of Bias (Based on Cochrane Handbook)	Setting	Sample Size (Treatment Group; Placebo Group)	Mean Age/ Range (Treatment Group; Placebo Group)	Diarrhea Definition	Antibiotic (s)	Time From Antibiotic to Probiotic, d	Probiotic Species	Dosage Per Day	Probiotic Duration (d)	Follow-up Period (From the Cessation of Antibiotics Treatment)
Selinger et al[39]	Unclear	Adults, in-patient	117/112	57.9/57.0	Other definition	Various	2	A combination of *B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*	9×10^{11} CFU	Various, AC +7	4 wk
Shavakhi et al[40]	Low	Adults	90/90	42.3/42.2	NR	*H. pylori* eradication	0	A combination of *L. casei, L. rhamnosus, L. acidophilus*, and *L. bulgaricus, B. breve* and *B. longum,* and *Streptococcus thermophiles*	2×10^{8} CFU	14 d, AC	4 wk
Francavilla et al[41]	Low	Adults, dyspepsia	44/43	49/44	NR	*H. pylori* eradication	0	A combination of 2 strains of *L. reuteri*	2×10^{8} CFU	7 d, AC	61 d
Ouwehand et al[42]	Low	Adults, in-patient	336/167	49.9/50.0	WHO*	One of broad-spectrum penicillin, cephalo-sporin, or clindamycin	1.5	A combination of *L. acidophilus, L. paracasei* and *B. lactis*	4.17×10^{9} or 1.70×10^{10} CFU	10-21 d, AC+7	4 wk
Helps et al[43]	Low	Adults, in-patient	44/41	62.27/62.49	WHO*	Various	2	*L. casei, Shirota*	1.3×10^{10} CFU	Various, AC +7	12 wk after recruitment
Wright et al[44]	Low	Adults, in-patient	41/46	85.4/86.1	Adjusted WHO†	Various	NA	*L. casei, Shirota*	130 mL	Various, AC	4 wk after recruitment
Ehrhardt et al[45]	Unclear	Adults, in-patient	246/231	60.1/56.5	WHO*	Various	2	*S. boulardii*	3.6×10^{10} CFU	Various but <8 wk, AC+7	7 wk
Evans et al[46]	Low	Adults, healthy volunteers	80/80	34.6/33.9	Other definition	Amoxicillin-clavulanic acid	0	A combination of *L. helveticus* and *L. rhamnosus*	$0{\cdot}4\times10^{9}$ and 7.6×10^{9} CFU, respectively	14 d, AC+7	8 wk

Shafaghi et al[47]	High	Adults	38/38	43.75/43.35	NR	*H. pylori* eradication	3	A combination of *L. casei, L. rhamnosus, Streptococcus thermophilus, B. breve, L. acidophilus, B. longum, L. bulgaricus*	4×10^8 CFU	17 d, 3 days earlier+AC	1 wk
Chotivitaya-tarakorn et al[48]	Unclear	Adults, dyspepsia	54/54	54.15	NR	*H. pylori* eradication	0	*S. boulardii*	565 mg	7 or 14 d, AC	2-3 wk
Haghdoost et al[49]	Unclear	Adults, dyspepsia	88/88	28.34	NR	*H. pylori* eradication	0	A combination of *L. actobacillus* and *Bifidobacterium*	3×10^9 CFU	38 d, AC+28	10 wk
Jiang and Zhu[50]	Unclear	Adults	111/111	35.2/34.8	NR	*H. pylori* eradication	0	*Bifidobacterium*	6 capsules	14 d, AC	4 wk
Trallero et al[51]	Unclear	Adults	18/18	38.5	Other definition	Amoxicillin-clavulanic acid	0	A combination of *L. acidophilus, L. rhamnosus, B. breve, B. longum, B. lactis* and *B. bifidum*	1×10^9 CFU	30 d, AC+22	22 d
Romeo et al[52]	Unclear	Adults	74/73	18-65	WHO*	Amoxicillin/ clavulanic acid	0	Combination including *Lactobacillus GG*	Unclear	7 d, AC	0
Rajkumar et al[53]	Unclear	Adults, in-patient	549/577	73.7/73.5	Other definition	Various	2	A combination of *L. casei, L. delbrueckii subspecies bulgaricus* and *S. thermophilus*	2×10^{10}, 2×10^8, and 2×10^8 CFU, respectively	Various, AC +7	3 wk

*WHO, diarrhea was defined as ≥3 loose stools within a 24-hour period.
†Adjusted WHO, diarrhea was defined as ≥3 loose stools/day for at least 2 days.
AC indicates antibiotic course; NR, not reported; WHO, World Health Organization.

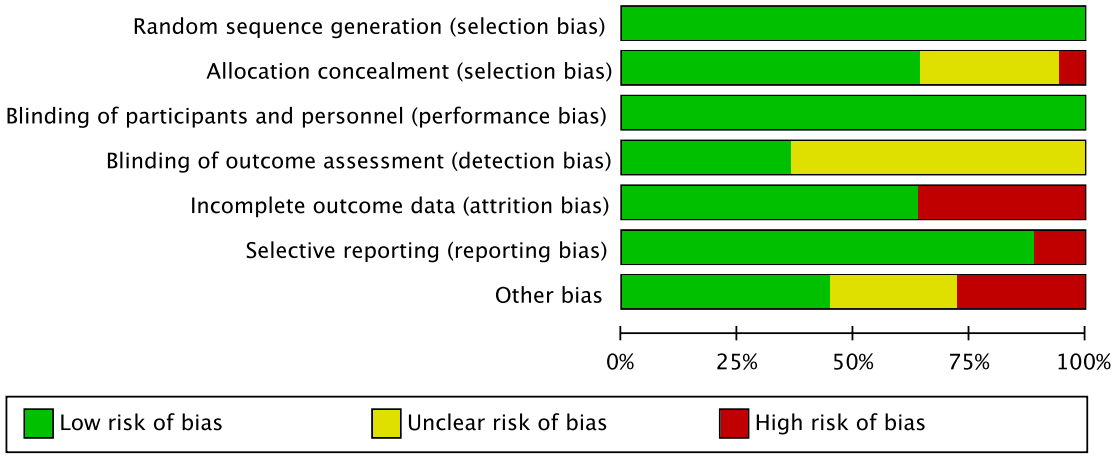

FIGURE 2. Risk of bias. full color online

products under different conditions through the most comprehensive subgroup analyses.

METHODS

This meta-analysis was conducted strictly following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[8]

Selection Criteria

Inclusion criteria: (1) patients limited to the adults both inpatients and outpatients who were prescribed antibiotics for various reasons with probiotics (experimental groups) or placebo (control group); (2) providing the occurrence of AAD; and (3) the study designed as an RCT.

Exclusion criteria: (1) duplicate studies, animal researches, preclinical studies, and case reports; (2) not-blinded trials; (3) unknown probiotics composition; and (4) existing diarrhea in baseline or containing laxative-related diarrhea.

Literature Search

The databases involving the PubMed, EMBASE, Web of Science, and Cochrane Library were searched for the RCTs on probiotics to prevent AAD. Publications in any language from the databases inception to February 2020 were included. The search terms were the combinations of the following Mesh terms and key words: "probiotic(s)," "diarrhea," "anti-bacterial agents," "antibiotic(s)," "antibiotic-associated diarrhea," "placebo," "randomized," and "randomized controlled trial."

Data Extraction and Quality Assessment

The data extraction was conducted using the standardized form by 2 independent researchers (W.L. and Q.Z.). The primary outcome was the occurrence of AAD during the follow-up period. The secondary outcome was the incidence of adverse events. Other data extracted included demographics, participant setting, indications for antibiotics, probiotics species and dosage, probiotics duration, time from antibiotics to probiotics, follow-up period, and diarrhea definition.

The Cochrane Handbook for Systematic Reviews of Interventions[9] was applied to assess the quality of the selected studies. Two researchers assessed the eligibility and quality of each article independently. Any discrepancies were resolved through consensus, adjudicated with the support of a third investigator.

Statistical Analyses

We used the RevMan V.5.2[10] and Stata Release V.15.1 (StataCorp, College Station, TX) to perform the data analyses. The pooled relative risk (RR) and the 95% confidence interval (CI) were determined by a random-effects model (DerSimonian-Laird method[11]) or a fixed-effects model (Mantel-Haenszel method[12]). The χ^2 test and I^2 statistic were used to evaluate the heterogeneity of included studies.[13,14] $P < 0.1$ or $I^2 > 50\%$ indicated substantial heterogeneity and a random effect would be adopted. Otherwise, a fixed-effects model would be applied. Sensitivity analysis and subgroup analyses were carried out to explore the sources of heterogeneity. In addition, we assessed the publication bias by the funnel plot, Begg test, and Egger tests.[15–17]

RESULTS

Eligible Studies

A systematic search conducted in February 2020 identified 1789 citations (PubMed 204, Cochrane Library 439, EMBASE 533, and Web of Science 613). Of these studies, 36 RCTs[18–53] with 9312 subjects met the inclusion criteria (35 published in English and one in Spanish). Details of the search flow are depicted in Figure 1. The probiotics species studied in the trials primarily included *Lactobacillus*, *Saccharomyces*, *Bifidobacterium*, and *Streptococcus*. Probiotics were used at the same time as antibiotics or were prolonged by 2 to 28 days after the therapy. Diarrhea was defined by the World Health Organization (WHO) criterion in 8 studies ($\geq$3 loose stools within a 24-h period).[54] Six studies applied an adjusted WHO criterion ($\geq$3 loose or liquid stools/day for at least 2 d). Other RCTs defined diarrhea based on the number of bowel movements per day and the consistency of the stool. Table 1 summarizes the details of participants and intervention.

Quality Assessment

The quality assessment results are shown in Figure 2, whereas Figure 3 displays the risk of bias of individual study. Among the eligible studies, 13 RCTs were triple-blinded, and the reminders were not clearly reported about

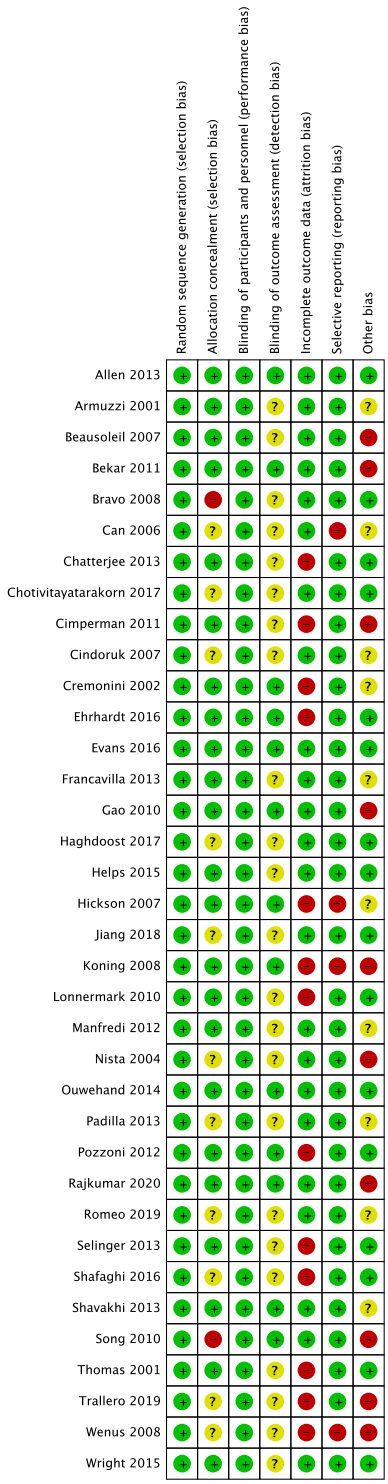

FIGURE 3. Risk of bias summary: green, low risk; yellow, unclear risk; red, high risk.

the detection bias. Attrition bias and other biases were assessed to be higher for lacking an intention-to-treat analysis (11/36), excessive or unbalanced loss of follow-up (6/36), funding bias (4/36), small sample size (3/36), unbalanced baseline (3/36), or short follow-up period (1/36).

Overall Effect of Probiotics

As substantial heterogeneity was observed among the included studies ($P < 0.1$, $I^2 = 58\% > 50\%$), we calculated the overall AAD rate using a random effect model. Probiotics reduced the incidence of AAD by 38% (RR, 0.62; 95% CI, 0.51-0.74) in comparison with placebo (Fig. 4).

Sensitivity Analyses and Subgroup Analyses

Sensitivity analysis revealed that the pooled RR of probiotic effectiveness was robust. No single study significantly affected the overall effect.

Based on the characteristics of the studies, such as the quality of publications, age, participant setting, dosage, and intervention duration, we carried out a series of subgroup analyses. There were significant differences ($P < 0.1$) among the 4 subgroups including reasons for antibiotics treatment ($P = 0.0007$), probiotic duration ($P = 0.006$), probiotic dosage ($P = 0.05$), and time from antibiotic to probiotic ($P = 0.03$).

Thirteen studies during *Helicobacter pylori* eradication had a higher efficacy than those used antibiotics for other reasons (RR, 0.36; 95% CI, 0.25-0.53; $I^2 = 31\%$ vs. RR, 0.75; 95% CI, 0.63-0.90; $I^2 = 49\%$).

Probiotic duration equal to the antibiotics course is more effective than prolonging at least 7 days after the end of antibacterial treatment (RR, 0.42; 95% CI, 0.31-0.58; $I^2 = 10\%$ vs. RR, 0.74; 95% CI, 0.58-0.95; $I^2 = 55\%$).

The daily dose of probiotics $<10^{10}$ CFU is more effective for preventing AAD (RR, 0.49; 95% CI, 0.33-0.72; $I^2 = 43\%$ vs. RR, 0.77; 95% CI, 0.60-0.98; $I^2 = 52\%$).

Using probiotics within the first 2 days of antibiotic treatment is more beneficial to prevent diarrhea (RR, 0.54; 95% CI, 0.43-0.67; $I^2 = 43\%$ vs. RR, 0.79; 95% CI, 0.60-1.03; $I^2 = 52\%$).

Other subgroups, as shown in Table 2, were also evaluated but were not statistically different.

Adverse Events

A total of 15 studies described adverse events, mainly involving nausea, bloating, and dyspepsia. Four of them reported no adverse events either in the probiotics group or in the placebo, and 2 registered serious adverse events but not attributable to probiotics. There were no statistically significant increased adverse events in the probiotics group (RR, 1.00; 95% CI, 0.87-1.14; $P = 0.97$) (Fig. 5).

Publication Bias

The funnel plot, Begg test, and Egger test were applied to assess the publication bias of the enrolled studies. These results provided evidence of publication bias (Begg test: $z = 2.36$, $Pr > |z| = 0.018 < 0.05$; and Egger test: $t = -4.77$; 95% CI, −2.40 to −0.97; $P < 0.05$). We use the trim and fill method to correct the publication bias and yielded the same pooled RR of 0.62 as initial outcomes, which suggested that results of the overall effect were stable, and publication bias had few effects on the results. Therefore, our asymmetric funnel plot may be caused by other reasons such as studies with low quality or small sample size (Fig. 6).

DISCUSSION

Our meta-analysis indicated a reduction of AAD from 16% in placebo to 13% in probiotic-treated groups (RR, 0.62; 95% CI, 0.51-0.74; random-effects). Further subgroup analyses suggested that the protective effect was still significant when grouped by reasons for antibiotics treatment,

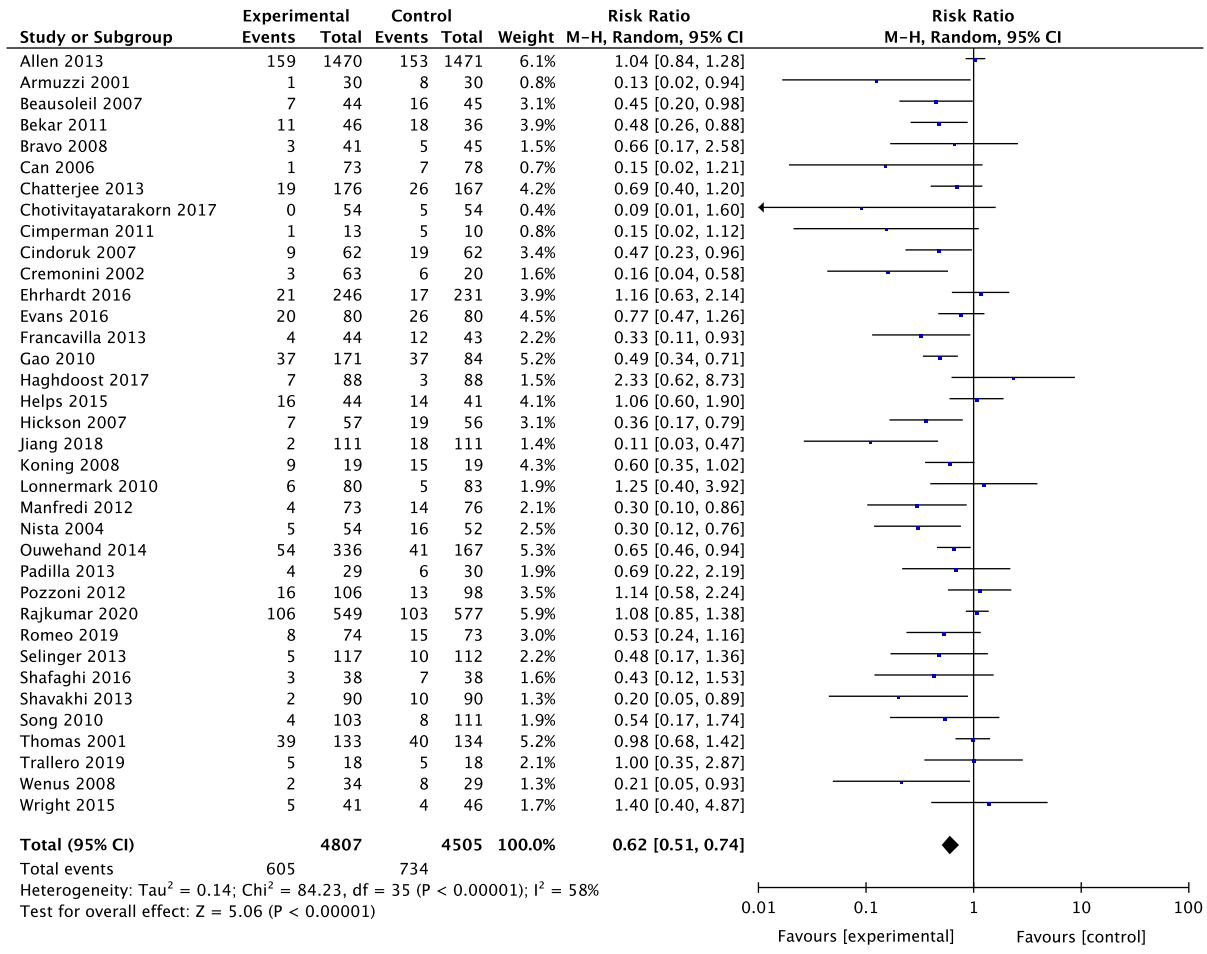

FIGURE 4. Forest plot for the overall effect of probiotics. full color online

probiotic duration, probiotic dosage, and time from antibiotic to probiotic.

Compared with antibiotics treatments for other reasons, probiotics showed more effective protection during *H. pylori* treatment. Certain probiotics, when used as an auxiliary in *H. pylori* eradication, can increase the eradication rate and reduce side effects.[55] Meta-analyses for *Saccharomyces boulardii* and *Lactobacillus* both showed statistically promising results. *S. boulardii* significantly improved the eradication rates (RR, 1.11; 95% CI, 1.06-1.17) and reduced the incidence of diarrhea (RR, 0.51; 95% CI, 0.42-0.62).[56] So was the *Lactobacillus* (improving eradication rates: OR, 1.78; 95% CI, 1.21-2.62; reducing incidence of diarrhea: OR, 0.23; 95% CI, 0.11-0.48).[57] In terms of the mechanism of probiotics in *H. pylori* eradication, animal investigations have indicated that probiotics may regulate immune activity by controlling cytokine and inflammatory/anti-inflammatory chemokine balance, such as interleukin-8 and secretory immunoglobulin A, thereby reducing gastric activity and inflammation. Also, probiotics assisted in promoting the *H. pylori* eradication through a physiological or nonspecific mechanism. Certain probiotics directly or in combination with their products stimulated gastric epithelium to produce antibacterial peptides, inhibited the growth of *H. pylori* by secreting short-chain fatty acids, competitively inhibited the adhesion of pathogens to the gastric mucosal layer, improved the epithelial barrier function, and increased mucin production.[58]

We also explored the dose effect of probiotics in our meta-analysis. Our results showed that high-dose probiotics ($\geq 10^{10}$ CFU/d) were statistically less effective than low-dose probiotics ($P = 0.05 < 0.10$). However, a previous meta-analysis conducted by Johnston et al (involving adults and children) demonstrated that higher dosage ($> 10^{10}$ CFU/d) had a more effective trend than lower dosage but not significantly (RR, 0.34; 95% CI, 0.23-0.49 vs. RR, 0.61; 95% CI, 0.08-4.60; $P = 0.57 > 0.10$).[59] This may be because we excluded children and the difference in sample size between subgroups. Hence, more RCTs on dose-response were needed to determine whether probiotics in higher doses were more effective and safe.

Our results are almost consistent with the previous meta-analysis in terms of the duration and starting time of probiotics.[60,61] It is beneficial to use probiotics as early as possible to maintain the gut flora's stability and prevent the overgrowth of pathogens. Concerning the optimal duration of probiotics, we suggested that probiotics use during antibiotic therapy can effectively prevent AAD. However, whether it is necessary to prolong the use of probiotics after the end of antibiotic treatment still needs more clinical evidence and theoretical support.

Twelve studies applied *Lactobacillus* as intervention indicated a more protective trend among all the probiotics species (RR, 0.67; 95% CI, 0.50-0.91). Among them,

TABLE 2. The Results of Subgroup Analyses

Subgroup	No. Trials	Effect Estimate		Heterogeneity Test	
		Risk Ratio	95% CI	*P*, *P*	*P* for Interaction
Overall effect	36	0.62	0.51-0.74	58%, <0.1	—
Risk of bias					
Low risk	13	0.72	0.55-0.93	59%, 0.003	0.25
Unclear risk	18	0.57	0.42-0.77	63%, 0.0002	
High risk	5	0.45	0.27-0.76	0%, 0.82	
Diarrhea definition					
WHO definition	8	0.74	0.55-0.99	64%, 0.007	0.27
Adjusted WHO definition	6	0.64	0.37-1.11	30%, 0.21	
Others	22	0.53	0.40-0.70	63%, <0.01	
Reasons for antibiotics treatment					
For *H. pylori* eradication	13	0.36	0.25-0.53	31%, 0.13	0.0007
For other reasons	23	0.75	0.63-0.90	49%, 0.005	
Participant setting					
Hospital	16	0.75	0.60-0.94	61%, 0.0007	0.64
Community	4	0.69	0.51-0.92	0%, 0.92	
No. antibiotics					
One	8	0.62	0.52-0.75	0%, 0.84	0.68
Others	28	0.58	0.45-0.75	64%, <0.01	
Probiotic duration					
During antibiotics treatment	12	0.42	0.31-0.58	10%, 0.34	0.006
At least 1 week after antibiotics	16	0.74	0.58-0.95	55%, 0.004	
No. probiotics species					
One	15	0.64	0.44-0.93	56%, 0.004	0.86
Mixture	20	0.61	0.49-0.76	60%, 0.0003	
Probiotic dosage (CFU/d)					
$\geq 10^{10}$	14	0.77	0.60-0.98	52%, 0.01	0.05
$< 10^{10}$	12	0.49	0.33-0.72	43%, 0.06	
Follow-up duration (from the cessation of antibiotics treatment) (wk)					
≥4	14	0.64	0.47-0.86	64%, 0.0006	0.45
<4	20	0.54	0.41-0.72	57%, 0.0008	
Probiotic species					
Lactobacillus	12	0.67	0.50-0.91	44%, 0.05	0.10
S. boulardii	6	0.69	0.39-1.22	47%, 0.09	
Lactobacillus+Bifidobacterium	6	0.82	0.57-1.17	56%, 0.04	
Other (mixed) species	12	0.41	0.27-0.63	71%, <0.01	
Time from antibiotic to probiotic (d)					
<2	22	0.54	0.43-0.67	43%, 0.02	0.03
2-7	13	0.79	0.60-1.03	52%, 0.01	

H. pylori indicates Helicobacter pylori; S. boulardii, Saccharomyces boulardii.

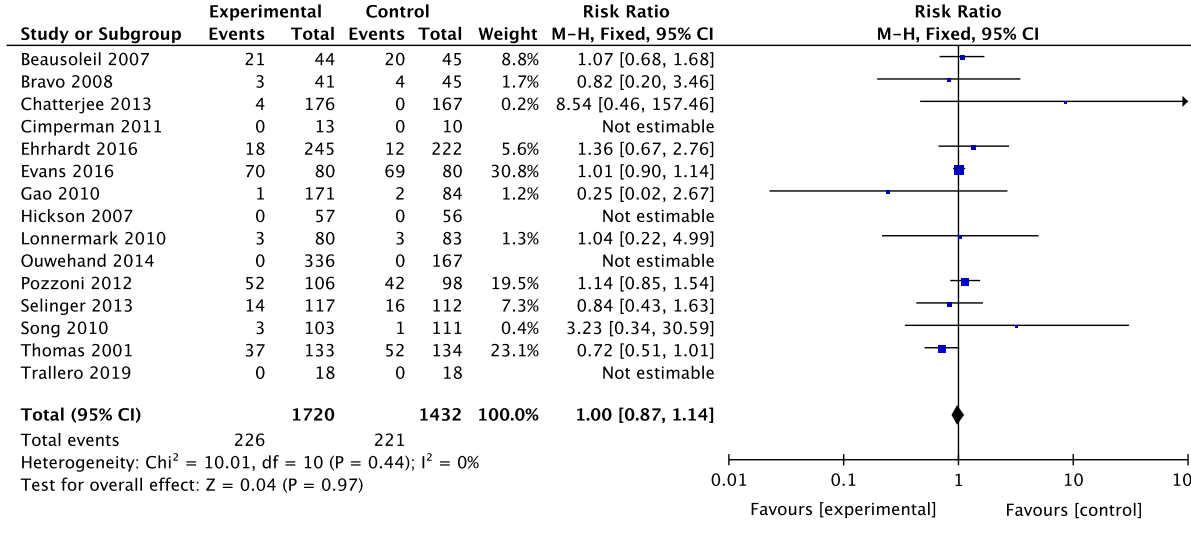

FIGURE 5. Forest plot of adverse events. full color online

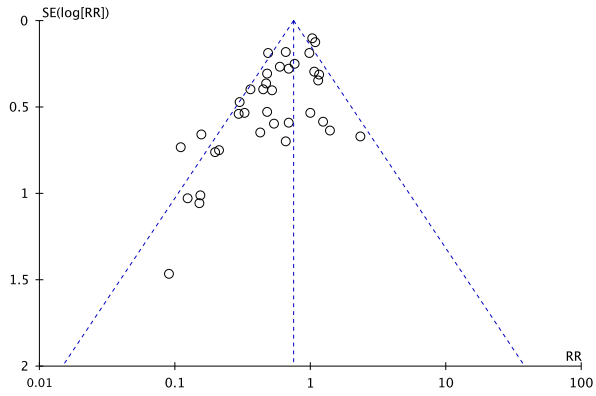

FIGURE 6. Funnel plot of publication bias. RR indicates relative risk; SE, standard error. full color online

L. rhamnosus GG (LGG) is the most studied. A meta-analysis proposed that LGG significantly reduced the risk of diarrhea (RR, 0.49; 95% CI, 0.29-0.83).[62] This effect may be related to the colonization of LGG in the intestine. It not only enhances the survival rate of the intestinal epithelium survival and preserves cytoskeletal integrity, but also secretes lectin-like proteins 1 and 2 to resist biofilms produced by various pathogens.[63] Unfortunately, because of the insufficient sample size, some probiotics strains cannot be analyzed separately. In addition, we did not find significant differences in the efficacy of single species and multiple species (RR, 0.64; 95% CI, 0.44-0.93 vs. RR, 0.61; 95% CI, 0.49-0.76; $P = 0.86 > 0.1$).

The type of antibiotic was reported as the strongest predictor for AAD. Although ampicillin/amoxicillin, cephalosporins, and clindamycin used alone were most frequently associated with AAD, other antibiotics, when used in combination, also increased the risk of AAD.[64] Unfortunately, many RCTs did not register specific antibiotics, which prevented us from performing subgroup analysis.

We extracted the data related to adverse events from 15 studies and thus calculated the pooled RR of 1.00 with no statistical significance (95% CI, 0.87-1.14; $P = 0.97$). A comprehensive systematic review on probiotics safety based on 622 studies displayed a pooled RR of 1.00 (95% CI, 0.93-1.07; $P = 0.999$), which was close to our finding.[65] These pieces of evidence were sufficient to show that short-term use of probiotics would not bring about serious side effects on a population without severe systemic disease or immunodeficiency. However, specific patients, including critical illness, using a central venous catheter, immunosuppression, should be sensitive to the adverse effects.[66] Some case reports and clinical studies have reported probiotics-related adverse events involving systemic infections, gastrointestinal side effects, deleterious metabolic activities, and gene transfer.[67] In short, probiotics are safe to use in preventing AAD.

There were some limitations. First, some heterogeneity was observed in our results. Both the subgroup analyses and sensitivity analysis failed to explain the source of heterogeneity. Second, some included studies failed to mention all specific characteristics. Thus, several subgroup analyses could not enroll all the 36 RCTs.

Nevertheless, our research also had some advantages. We adopted rigorous inclusion criteria to collect more representative data. During the citations identified, we excluded 2 publications with unknown probiotics composition. To avoid interference with baseline conditions, RCTs that included existing diarrhea or containing laxative-related diarrhea were also excluded. In addition, we conducted subgroup analyses as comprehensive as possible, and the trend of probiotics in some specific situations had been explored.

Our study suggests that using probiotics within 2 days during antibiotic treatment significantly reduces the incidence of AAD in adults and is safe. Besides, the existing evidence showed that *S. boulardii* supplementation or *Lactobacillus* supplementation in *H. pylori* eradication therapy significantly increased the eradication rate and reduced the incidence of diarrhea. But the role of other probiotics in *H. pylori* eradication had not yet been fully clarified. Of course, to match the population included in this meta-analysis, these findings are restricted to adults without immunodeficiency and the history of intensive care unit.

CONCLUSIONS

Our meta-analysis suggested that during antibiotic treatment, taking probiotics as early as possible has a positive and safe effect on preventing antibiotic-related diarrhea in adults. However, further studies should focus on the optimal dosage and duration of probiotics and pay attention to the strain specificity to develop a specific recommendation.

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