# Comparative efficacy and tolerability of probiotics for antibiotic-associated diarrhea: Systematic review with network meta-analysis

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## Abstract

**Background:** Probiotics are commonly used for the prevention of antibiotic-associated diarrhea (AAD). However, the optimum regimen remains controversial.

**Objective:** The objective of this article is to compare and rank the relative efficacy and tolerability among all available probiotic agents for AAD through a network meta-analysis.

**Methods:** Eligible studies were identified by searching PubMed, Embase, Medline, Cochrane library and Web of Science for randomized controlled trials (RCTs) that examined the efficacy of probiotic therapy for AAD. A random-effects model was applied within a frequentist framework. Quality of evidence was performed by the GRADE approach. The project was prospectively registered with PROSPERO (CRD 42016050776).

**Results:** Fifty-one articles (60 comparisons, 9569 participants), including 10 probiotic interventions, were identified. *Lactobacillus rhamnosus* GG (LGG) had the highest probability of being ranked best both in effectiveness (odds ratio (OR), 95% confidence interval (CI) = 0.28 (0.17, 0.47)) and tolerance (0.44 (0.23, 0.84)) on prevention of AAD. With regard to reducing *Clostridium difficile* infection rate, *Lactobacillus casei* (*L. casei*) was considered better efficacy (0.04 (0.00, 0.77)) and medium tolerance (0.56 (0.19, 1.66)). Strain combination reported no superiority over single strain in either efficacy or tolerability.

**Conclusions:** LGG is probably the best option to consider when AAD is indicated. *L. casei* appears to be the most efficacious choice when associated with severe *C. difficile*-related cases.

## KEY SUMMARY

- **Summarize the established knowledge on this subject**
- The prevalence of antibiotic-associated diarrhea (AAD) has been increasing over the last decades owing to the widespread use of antibiotics.
- Several probiotic regimens have been utilized in clinical practice for the management of AAD; however, the optimum regimen remains controversial.
- **What are the significant and/or new findings of this study?**
- *Lactobacillus rhamnosus* GG is probably the best option to consider when AAD is indicated.

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- *Lactobacillus casei* appears to be the most efficacious choice when associated with severe *Clostridium difficile*-related cases.
- Strain combination reported no superiority over single strain alone in either efficacy or tolerability for patients with AAD.

## Introduction

Antibiotic-associated diarrhea (AAD), emerging as a common complication of antibiotic use, is defined as unexplained diarrhea that occurs in association with antibiotics.[1] The prevalence of AAD ranges from 5% to 49%, occurring at any point from the initiation of therapy to two months after antibiotic exposure.[2] Disruptions of indigenous gastrointestinal microbiota and mucosal integrity, overgrowth of pathogens, and metabolic imbalances have been considered as major mechanisms involved in the pathogenesis of AAD.[3,4] Typically, AAD is brief and self-limiting with no specific pathogenic agent identified; however, *Clostridium difficile* is responsible for the most severe cases in 10%~25% of all episodes of AAD, which may lead to electrolyte disturbances, pseudo-membranous colitis, toxic megacolon and, rarely, death.[5,6] Risk factors such as prolonged use of broad-spectrum antibiotics, old age, extended hospitalization, oral administration, and comorbidities are all proposed to influence the incidence and development of AAD.[5,7,8] Antibiotics such as broad-spectrum β-lactams, glycopeptides and fluoroquinolones that act on anaerobes are most commonly linked to AAD.[9] Current standard treatments for AAD have limitations. Mild cases have no established therapy, and are customarily treated with discontinuation of the antibiotic, supportive care and dietary changes. However, serious cases often require bed rest, intravenous fluids, and additional antibiotics such as metronidazole or vancomycin, and may relapse in almost 25% patients.[10]

Probiotics, defined as non-pathogenic living microorganisms, when ingested in adequate amounts, may colonize the intestinal tract and promote microbiota restoration, conferring a health benefit on the host.[11] The rationale underlying probiotic administration is derived from normalization of unbalanced commensal gut microbiota, modulation of mucosal function and metabolic process, stimulating systemic immune response and suppressing pathogenic bacteria colonization, using specific probiotic strains.[12] Based on this, a variety of organisms, such as *Lactobacillus*, *Saccharomyces* and *Bifidobacterium*, have been recommended empirically in recent epidemiological studies, both during and after antibiotic intake for the management of AAD, with encouraging results.[1,6] However, it remains controversial which certain probiotic strains are more effective and also tolerable, or whether a multi-strain combination is superior to a single-strain probiotic preparation alone.

Previous traditional pairwise meta-analyses have generally estimated direct comparisons between probiotics, which undermined findings about relative efficacy and tolerance of interventions, because they can answer only questions about pairs.[13–15] Network meta-analysis is an extension of head-to-head meta-analysis that aims to combine direct and indirect evidence into a single effect size and rank all available treatments, calculating estimates for interventions even if they have not been compared directly in current research, provided that a common comparator exists.[16] In this study, therefore, we performed a systematic review and network meta-analysis of randomized controlled trials (RCTs) in order to comprehensively compare and rank the relative efficacy and tolerability among all available probiotic preparations for the prevention of AAD. Incidence of diarrhea and *C. difficile* infection rate were summarized to estimate treatment efficacy, while total occurrence of adverse events and occurrence of adverse event subtypes were adopted for tolerability assessment.

## Methods

Our study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the Cochrane Collaboration recommendations.[17,18] The project was prospectively registered with the PROSPERO database of systematic reviews (CRD 42016050776).[19]

### Search strategy

For this network meta-analysis, an experienced medical investigator (ZCY) searched PubMed, Embase, Medline, Cochrane library and Web of Science for RCTs published from January 1, 1996 to December 31, 2016 that examined the efficacy of probiotic therapy for AAD. Publications with the following search terms and Medical Subject Headings (MeSH) headings were included: ((probiotic OR *Lactobacillus*) OR *Bifidobacterium*) OR *Saccharomyces*) OR yogurt) AND antibiotic AND diarrhea. No language restriction was imposed. Major scientific websites and conference abstracts or presentations were also considered. Reference lists of the obtained papers, relevant reviews and meta-analyses were retrieved manually to identify articles not included in the preliminary searches.

## Selection criteria

The following studies were eligible for inclusion: RCTs irrespective of language, in which an identified probiotic agent was compared to active or placebo control; participants were individuals who received oral antibiotic therapy for any reason; the intervention was oral probiotics intake in either supplements (e.g. capsule, sachet) or foods (e.g. yogurt) during or after oral antibiotic administration, with any duration and dose; the incidence of diarrhea, as defined by the individual studies, was listed as one of the major outcomes; articles were published in the last 20 years.

## Data extraction and quality assessment

Three reviewers (CJY, ZCY and ZYQ) independently screened citations for eligibility by title, abstract and full text if necessary, evaluated methodological quality, and extracted the relevant data from the enrolled studies with a predesigned Excel spreadsheet, including general details of the study, participants' information, characteristics of the treatments and main outcome measures. Risk of bias of individual studies was assessed with the Cochrane Collaboration tool in seven specified domains.[18] Each domain was classified as "low risk," "unclear" or "high risk." Discrepancies among investigators during screening, data extraction and quality assessment process were resolved by joint review.

## Statistical analyses

Direct comparisons of therapies were estimated by traditional approach. Since all data were expressed as dichotomous endpoints, we summarized the estimates as odds ratio (OR), with 95% confidence intervals (CIs).[20, 21] To provide more conservative pooled effects, a random-effects model was performed. Heterogeneity among studies was estimated by the $I^2$ statistic and Cochran $Q$ test.[22, 23] A $p$ value less than 0.10 for the $Q$ test was considered as significant.[18] The restricted maximum likelihood (REML) method was applied to estimate the heterogeneity variance both in pairwise and network meta-analysis.[24] Publication bias was assessed examining comparison-adjusted funnel plots asymmetry[25] and Begg's and Egger's tests, where appropriate.[26]

In addition to the direct comparisons, we analyzed pooled data for probiotic treatments using random-effects models within a frequentist network meta-analysis. Pooled effect sizes were summarized as ORs, with 95% CIs. To summarize the efficacy and tolerability of each probiotic therapy, we estimated the absolute rates of various probiotic preparations as well. Relative

ranks were accessed based on surface under the cumulative ranking curve (SUCRA),[27] and the resultant rankings were presented graphically.[25] Network inconsistency was appraised by comparing the indirect and direct evidence using the design-by-treatment interaction model (Higgins model),[28] and the node-splitting approach for major outcomes.[29] Meta-regression analyses were applied to verify similarities among studies by adding another covariate, which enabled confounders to be taken into account and allowed estimation of the summary attributable fraction for a set of exposures. A $p$ value less than 0.05 was considered as significant.[30,31]

Quality of evidence contributing to each estimate was assessed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria. For each comparison, we rated the quality of evidence as "high," "moderate," "low" or "very low."[32]

Data synthesis was conducted using STATA software (version 14.0), Cochrane Collaboration review manager software (RevMan, version 5.3) and GRADEprofiler (version 3.5).

## Results

### Study characteristics and methodological quality

The literature search process is shown in Supplementary Material 1: Figure S1. From 3265 unique publications, we identified 51 articles (60 comparisons, 9569 participants, see Supplementary Material 2) meeting the inclusion criteria, which covered 10 different probiotic therapies listed in Table 1. Overall, 4819 participants were allocated to an active intervention and 4750 to placebo. Across trials, the average age of the participants was 43.2 years, the percentage of male patients was approximately 59.5% and mean sample size of each comparison arm was 205. The baseline characteristics of the enrolled records are presented in Supplementary Material 1: Table S1. A graph and summary of methodological quality assessment for individual studies are summarized in Supplementary Material 3: Figure S3 and Table S3.

### Treatment efficacy

In terms of the primary outcome, the incidence of diarrhea, and the efficacy of probiotics on AAD was assessed for 10 interventions, presented in 51 publications (60 comparisons, 9569 participants), and each probiotic therapy was estimated in at least one RCT. Of 55 possible comparisons between probiotic treatments, 14 (25.5%) were compared in the studies directly (Figure 1(a)). All the regimens reduced the incidence of

**Table 1.** General characteristics of probiotic therapies on prevention of antibiotic-associated diarrhea.

| Intervention abbreviations | General characteristics |
| --- | --- |
| Multi-genera II | Combinations of two types of genera (*Lactobacillus* + *Bifidobacterium*, *Lactobacillus* + *Streptococcus*, *Bifidobacterium* + *Streptococcus*, *Bifidobacterium* + *Clostridium*) |
| Multi-genera III | Combinations of three or more types of genera (*Lactobacillus* + *Bifidobacterium* + *Streptococcus*, *Lactobacillus* + *Bifidobacterium* + *Enterococcus*, *Lactobacillus* + *Bifidobacterium* + *Lactococcus* + *Saccharomyces* + *Leuconostoc*, *Lactobacillus* + *Bifidobacterium* + *Propionibacterium*) |
| LGG | *Lactobacillus rhamnosus* GG |
| *L. rhamnosus* | *Lactobacillus rhamnosus* species except for *Lactobacillus rhamnosus* GG |
| *L. casei* | *Lactobacillus casei* species |
| *L. acidophilus* | *Lactobacillus acidophilus* species |
| *L. reuteri* | *Lactobacillus reuteri* species |
| *L. plantarum* | *Lactobacillus plantarum* species |
| *B. clausii* | *Bacillus clausii* species |
| *S. boulardii* | *Saccharomyces boulardii* species |

diarrhea; however, only six probiotic therapies showed superior efficacy compared with drug placebo in the network meta-analysis, those being LGG (0.28 (0.17, 0.47)), *L. casei* (0.29 (0.13, 0.68)), *B. clausii* (0.33 (0.11, 0.99)), *S. boulardii* (0.41 (0.29, 0.57)), *L. acidophilus* (0.57 (0.43, 0.76)) and Multi-genera II (0.60 (0.45, 0.81)), respectively (Figure 1(b)). Table 2 presents the efficacy results of each intervention compared with placebo. The incidence of diarrhea of any regimen ranged from 5% to 36% for all options. Ranking on the incidence of diarrhea, LGG was the highest one, followed by *L. casei, L. plantarum, S. boulardii, L. acidophilus*, *L. rhamnosus, B. clausii, L. reuteri*, Multi-genera II, Multi-genera III and placebo (Table 2). Despite *L. plantarum* therapy ranking in third place, information on the intervention was offered by only one report. The GRADE summary of findings for rating for all comparisons on incidence of diarrhea is summarized in Table 2 and Supplementary Material 4: Table S4.1; the quality of evidence was rated as moderate. Heterogeneity in the traditional meta-analysis was generally moderate (see Supplementary Material 4: Table S4.2 and Table S4.3).

Secondary outcomes comprised three indicators: *C. difficile* infection rate, fever rate and dehydration rate. In terms of *C. difficile* infection rate, 21 publications (21 comparisons, 6623 participants) and eight probiotic regimens were analyzed. Of 36 possible comparisons between interventions, eight (22.2%) were compared directly in the reports (Figure 1(c)). Apart from *L. plantarum*, all probiotics indicated superior efficacy, and

significant reductions were observed in two probiotic therapies (*L. casei* (0.04 (0.00, 0.77)) and *L. acidophilus* (0.25 (0.12, 0.52))), when compared with the placebo arm (Figure 1(d)). Ranking *C. difficile* infection rate, *L. casei* was the highest one, followed by *L. acidophilus*, Multi-genera III, *L. rhamnosus*, LGG, *S. boulardii*, Multi-genera II, *L. plantarum* and placebo (Table 2). Quality of evidence was rated as moderate for *C. difficile* infection rate (Table 2). Significant reduction was observed in fever rate (0.57 (0.35, 0.90)) and dehydration rate (0.31 (0.15, 0.67)) when probiotic therapy was applied for AAD (see Supplementary Material 4: Figure S4.1).

## Treatment tolerability

*Total occurrence of adverse events.* Thirty-two trials were enrolled in contrast with total occurrence rates of adverse reactions among 10 probiotic therapies. Of 55 possible comparisons between interventions, 13 (23.6%) were compared directly in the reports (Figure 2(a)). In terms of all adverse reactions, only LGG (0.44 (0.23, 0.84)) and Multi-genera II (0.57 (0.35, 0.93)) were significantly better tolerated than drug placebo, while most probiotic treatments did not report superiority over the placebo arm (Figure 2(b)). Table 3 indicates the comparisons of all adverse effects for each probiotic regimen with placebo. Ranking total tolerance, *L. reuteri* was the highest one, followed by *L. plantarum*, LGG, *S. boulardii*, *L. casei, L. rhamnosus*, Multi-genera II, *L. acidophilus*,
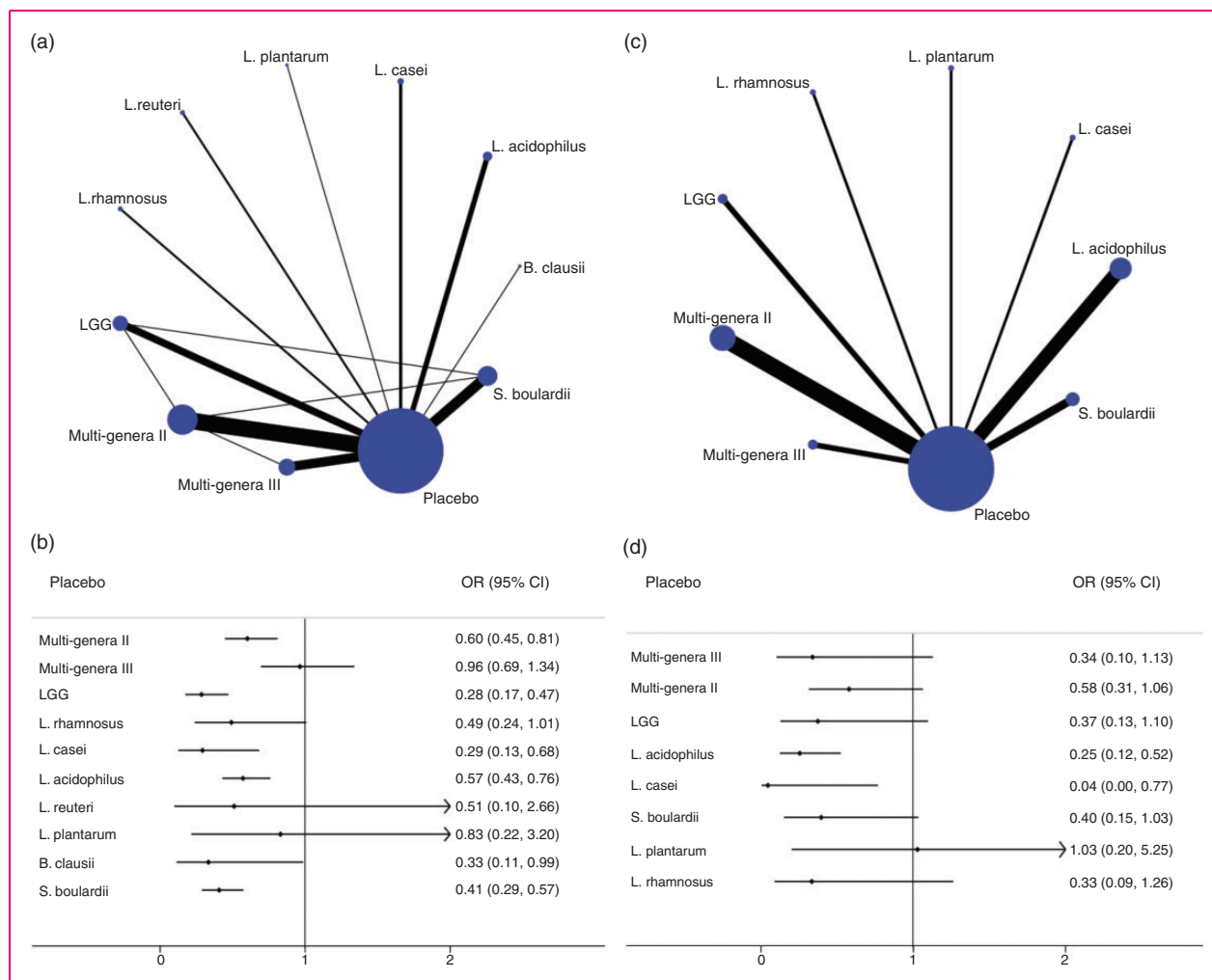
**Figure 1.** Network diagram and forest plot for treatment efficacy. (a) Network diagram of eligible comparisons for efficacy analysis of the incidence of diarrhea. The width of lines corresponds to the number of trials comparing every pair of interventions, and the size of nodes is proportional to the number of randomly assigned participants (indicates the sample size). (b) Forest plot of network meta-analysis for the incidence of diarrhea compared with placebo arm. (c) Network diagram of eligible comparisons for efficacy analysis of *Clostridium difficile* infection rate. The width of lines corresponds to the number of trials comparing every pair of interventions, and the size of nodes is proportional to number of randomly assigned participants (indicates the sample size). (d) Forest plot of network meta-analysis for *C. difficile* infection rate compared with placebo arm. LGG: *Lactobacillus rhamnosus* GG.

*B. clausii*, placebo and Multi-genera III (Table 3). The incidence rate of any adverse reactions of each regimen ranged from 1% to 37% for all interventions. Quality of evidence seemed to be low to moderate for total occurrence of adverse events (Table 3 and Supplementary Material 5: Table S5.1). All other comparisons and the data on traditional meta-analyses are available in Supplementary Material 5: Table S5.1 and Table S5.2.

*Occurrence of adverse event subtypes.* We summarized seven kinds of side effects for probiotics on prevention of AAD (Supplementary Material 5: Figure S5.1), and

five of them were processed by further network meta-analysis owing to limited sample size. Quality of evidence, relative ranks and occurrence rates of probiotic therapies in regard to subtypes of adverse reactions are listed in Supplementary Material 5: Table S5.3. In terms of constipation or vomiting, Multi-genera II might be a relatively optimal choice, although participants reported experiencing nausea more often. The lowest rate of abdominal/epigastric pain was reported with *L. reuteri*, while undergoing flatulence more often. Nausea was experienced less frequently with LGG, while patients experienced abdominal/epigastric pain more often. Other results of network and pairwise

**Table 2.** Quality of evidence, SUCRA values and effectiveness ranking for treatment efficacy.

| Interventions | Number of trials | Network meta-analysis: OR (95% CI) | Quality of evidence | Pairwise meta-analysis: OR (95% CI) | Quality of evidence | Absolute rates | SUCRA value | Mean rank[a] |
|---|---|---|---|---|---|---|---|---|
| (a) Incidence of diarrhea | | | | | | | | |
| Placebo | 56 | Reference | Reference | Reference | Reference | 0.18 | 5.0 | 10.5 |
| Multi-genera II | 9 | 0.66 (0.45, 0.81) | Low[b,c] | 0.71 (0.60, 0.83) | Low[b,c] | 0.14 | 31.0 | 7.9 |
| Multi-genera III | 16 | 0.96 (0.69, 1.34) | Moderate[b] | 0.94 (0.68, 1.31) | Moderate[c] | 0.11 | 15.8 | 9.4 |
| LGG | 6 | 0.28 (0.17, 0.47) | Moderate[b,e,g] | 0.26 (0.17, 0.40) | Moderate[b,g] | 0.05 | 83.1 | 2.7 |
| L. rhamnosus | 2 | 0.49 (0.24, 1.01) | Moderate[b] | 0.46 (0.24, 0.92) | Moderate[b,g] | 0.06 | 47.2 | 6.3 |
| L. casei | 3 | 0.29 (0.13, 0.68) | Moderate[b,g] | 0.27 (0.16, 0.46) | Moderate[b,g] | 0.06 | 80.8 | 2.9 |
| L. acidophilus | 5 | 0.57 (0.43, 0.76) | Moderate[b,e,g] | 0.46 (0.34, 0.61) | Moderate[b,g] | 0.21 | 55.7 | 5.4 |
| L. reuteri | 2 | 0.51 (0.10, 2.66) | Moderate[b,c,g] | 0.45 (0.16, 1.26) | Low[b,c,g] | 0.36 | 44.5 | 6.5 |
| L. plantarum | 1 | 0.83 (0.22, 3.20) | Low[b,e] | 0.82 (0.22, 3.14) | Moderate[b] | 0.05 | 76.2 | 3.4 |
| B. clausii | 1 | 0.33 (0.11, 0.99) | Moderate[b,g] | 0.29 (0.11, 0.77) | Moderate[b,g] | 0.10 | 45.8 | 6.4 |
| S. boulardii | 11 | 0.41 (0.29, 0.57) | Moderate[b,c,g] | 0.36 (0.28, 0.46) | Low[b,c] | 0.08 | 64.9 | 4.5 |
| (b) C. difficile infection rate | | | | | | | | |
| Placebo | 21 | Reference | Reference | Reference | Reference | 0.06 | 5.6 | 8.5 |
| Multi-genera II | 6 | 0.34 (0.10, 1.13) | Low[b,f] | 0.54 (0.35, 0.85) | Low[b,f] | 0.02 | 29.5 | 6.6 |
| Multi-genera III | 2 | 0.58 (0.31, 1.06) | Moderate[b,g] | 0.30 (0.10, 0.89) | Moderate[b,g] | 0.08 | 64.4 | 3.9 |
| LGG | 2 | 0.35 (0.10, 1.26) | Moderate[b,g] | 0.36 (0.14, 0.92) | Moderate[b,g] | 0.04 | 55.0 | 4.6 |
| L. rhamnosus | 1 | 0.32 (0.07, 1.49) | Moderate[b,g] | 0.35 (0.11, 1.12) | Low[b,e,g] | 0.02 | 56.8 | 4.5 |
| L. casei | 1 | 0.04 (0.00, 0.77) | Low[b,e,g] | 0.16 (0.06, 0.47) | Moderate[b,g] | 0.00 | 79.0 | 2.7 |
| L. acidophilus | 5 | 0.20 (0.08, 0.48) | Moderate[a,g] | 0.22 (0.13, 0.38) | Moderate[b,g] | 0.02 | 76.2 | 2.9 |
| L. plantarum | 1 | 1.03 (0.17, 6.32) | Low[b,e] | 1.03 (0.20, 5.24) | Low[b,e] | 0.04 | 29.3 | 6.7 |
| S. boulardii | 3 | 0.35 (0.15, 0.85) | Moderate[c,g] | 0.35 (0.19, 0.63) | Moderate[c,g] | 0.03 | 54.2 | 4.7 |

SUCRA: surface under the cumulative ranking curve; LGG: *Lactobacillus rhamnosus* GG; CI: confidence interval; OR: odds ratio.
[a]Rank was derived from the incidence of diarrhea values or *C. difficile* infection rate, 1 = best efficacy.
[b]Limitations in study design or execution (risk of bias). [c]Inconsistency in results. [e]Imprecision of results. [f]Publication bias. [g]Magnitude of effect.

meta-analysis are presented in Supplementary Material 5. A summary table of all endpoints for efficacy and tolerability is available in Supplementary Material 5: Table S5.4.

## Benefits versus harms

Figure 3 represented all probiotic therapies in order of relative efficacy ranks, indicating the individual contributions to the holistic results of effectiveness and tolerability. LGG, *L. casei, B. clausii, S. boulardii* and *L. acidophilus* were among the most effective interventions, whereas LGG performed better in tolerance. Each probiotic preparation was ranked according to both dimensions of efficacy and tolerability in Figure 4. With regards to the incidence of diarrhea, LGG, *S. Boulardii, L. rhamnosus* and *L. plantarum* were better in either efficacy or tolerability, as located in the upper right corner (Figure 4(a)). In terms of *C. difficile* infection rate, *L. casei* and *S. Boulardii* showed

better efficacy and tolerance, as shown in the upper right (Figure 4(b)).

## Meta-regression, modeling assumptions, and publication bias

Meta-regression with the primary outcome indicated no significant differences in mean age ($p = 0.17$), sex ratio ($p = 0.76$), recruiting area ($p = 0.45$), antibiotic in use ($p = 0.07$), indication ($p = 0.61$), dosage ($p = 0.45$) and duration ($p = 0.97$) of the probiotic therapy, which did not cause extensive changes in the results. Data on meta-regression are available in Supplementary Material 4: Table S4.4.

A design-by-treatment interaction model demonstrated no evidence for inconsistency in incidence of diarrhea ($p = 0.72$) and total occurrence of adverse events ($p = 0.35$). Similarly, no major inconsistency was identified by the node-splitting approach for major endpoints. As the sample size of trials for each
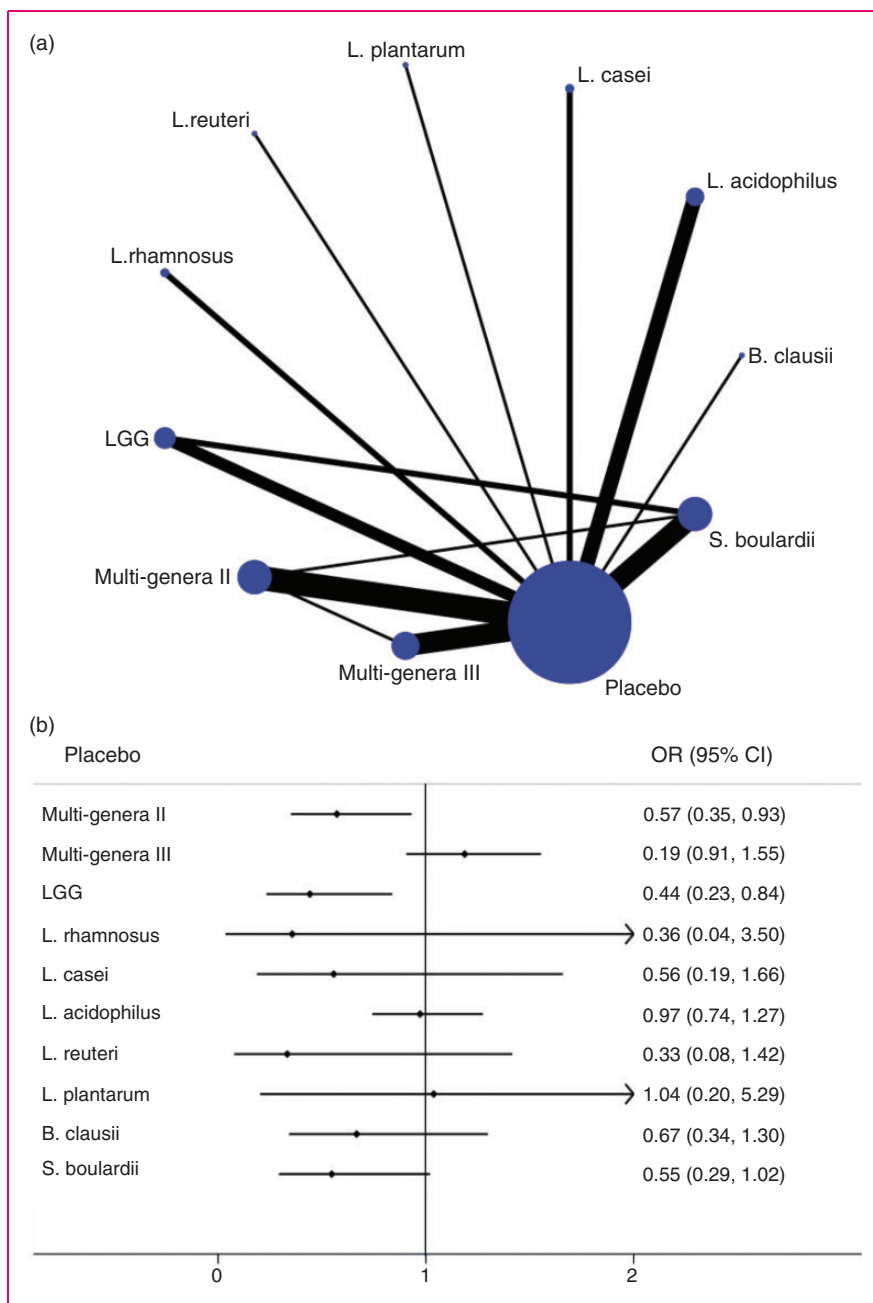
**Figure 2.** Network diagram and forest plot for treatment tolerability. (a) Network diagram of eligible comparisons for tolerability analysis of total occurrence of adverse events. The width of the lines corresponds to the number of trials comparing every pair of interventions, and the size of nodes is proportional to the number of randomly assigned participants (indicates the sample size). (b) Forest plot of network meta-analysis for total occurrence of adverse events compared with placebo arm. LGG: *Lactobacillus rhamnosus* GG.

group was limited, subgroup analysis was not further conducted.

Visual inspection of comparison-adjusted funnel plots for the major endpoints did not exhibit prominent asymmetry, and indicated no significant evidence of publication bias in the current meta-analysis (see Supplementary Material 4: Figure S4.2 and Supplementary Material 5: Figure S5.2).

## Discussion

In this network meta-analysis, we combined direct and indirect evidence from 51 RCTs (60 trials) involving 9569 participants with AAD to estimate the relative efficacy and tolerability of all currently available probiotic strategies. Overall, most probiotic treatments presented sufficient effect and good tolerance

**Table 3.** Quality of evidence, SUCRA and effectiveness ranking for total occurrence of adverse events.

| Interventions | Number of trials | Adverse event | | | | Mean occurrence rate of adverse events | SUCRA value | Mean rank[a] |
|---|---|---|---|---|---|---|---|---|
| | | Network meta-analysis: OR (95% CI) | Quality of evidence | Pairwise meta-analysis: OR (95% CI) | Quality of evidence | | | |
| Placebo | 37 | Reference | Reference | Reference | Reference | 0.22 | 20.2 | 9.0 |
| Multi-genera II | 8 | 0.57 (0.35, 0.93) | Low[b,c,f,g] | 0.80 (0.68, 0.94) | Low[b,c,f,g] | 0.16 | 46.0 | 6.4 |
| Multi-genera III | 6 | 1.19 (0.91, 1.55) | Moderate[b] | 1.33 (0.98, 1.80) | Moderate[b] | 0.29 | 6.4 | 10.4 |
| LGG | 5 | 0.44 (0.23, 0.84) | Moderate[b,g] | 0.27 (0.14, 0.52) | Moderate[b,g] | 0.12 | 69.3 | 4.1 |
| *L. rhamnosus* | 1 | 0.36 (0.04, 3.50) | Low[c,e,g] | 0.39 (0.05, 2.80) | Low[c,e,g] | 0.01 | 54.7 | 5.5 |
| *L. casei* | 2 | 0.56 (0.19, 1.66) | Moderate[c,g] | 0.48 (0.22, 1.04) | Moderate[e,g] | 0.15 | 59.3 | 5.1 |
| *L. acidophilus* | 5 | 0.97 (0.74, 1.27) | Moderate[b] | 0.95 (0.67, 1.34) | Moderate[b] | 0.37 | 41.5 | 6.9 |
| *L. reuteri* | 1 | 0.33 (0.08, 1.42) | Low[b,c,g] | 0.25 (0.06, 0.94) | Low[b,c,g] | 0.15 | 77.8 | 3.2 |
| *L. plantarum* | 1 | 1.04 (0.20, 5.29) | Low[b,e] | 1.04 (0.20, 5.28) | Moderate[b] | 0.04 | 73.8 | 3.6 |
| *B. clausii* | 1 | 0.67 (0.34, 1.30) | Moderate[b,g] | 0.42 (0.19, 0.91) | Moderate[b,g] | 0.44 | 37.9 | 7.2 |
| *S. boulardii* | 7 | 0.55 (0.29, 1.02) | Moderate[c,g] | 0.51 (0.36, 0.74) | Moderate[c,g] | 0.14 | 63.2 | 4.7 |

SUCRA: surface under the cumulative ranking curve; LGG: *Lactobacillus rhamnosus* GG; CI: confidence interval; OR: odds ratio.
[a]Rank was derived from occurrence rate values of adverse events for all studies, 1 = best tolerance.
[b]Limitations in study design or execution (risk of bias). [c]Inconsistency in results. [e]Imprecision of results. [f]Publication bias. [g]Magnitude of effect.



**Figure 3.** Network meta-analysis of major efficacy and tolerability. Probiotics were reported in order of efficacy ranking according to odds ratios (ORs). The column-defining treatment was compared with the row-defining treatment. For efficacy, an OR value below 1 favors the column-defining treatment; for tolerability, an OR value below 1 favors the row-defining treatment. Statistically significant results are shown in bold and underlined. LGG: *Lactobacillus rhamnosus* GG; CI: confidence interval.

compared with placebo. We made several key observations: (a) LGG had the highest probability of being ranked best in both effectiveness and tolerance for reducing the incidence of diarrhea in prevention of AAD, and significant benefits were noticed compared with placebo drug based on moderate quality of evidence; (b) with regard to reducing *C. difficile* infection rate, *L. casei* was considered to have better efficacy and tolerance based on moderate confidence in estimates; (c) strain combination reported no superiority
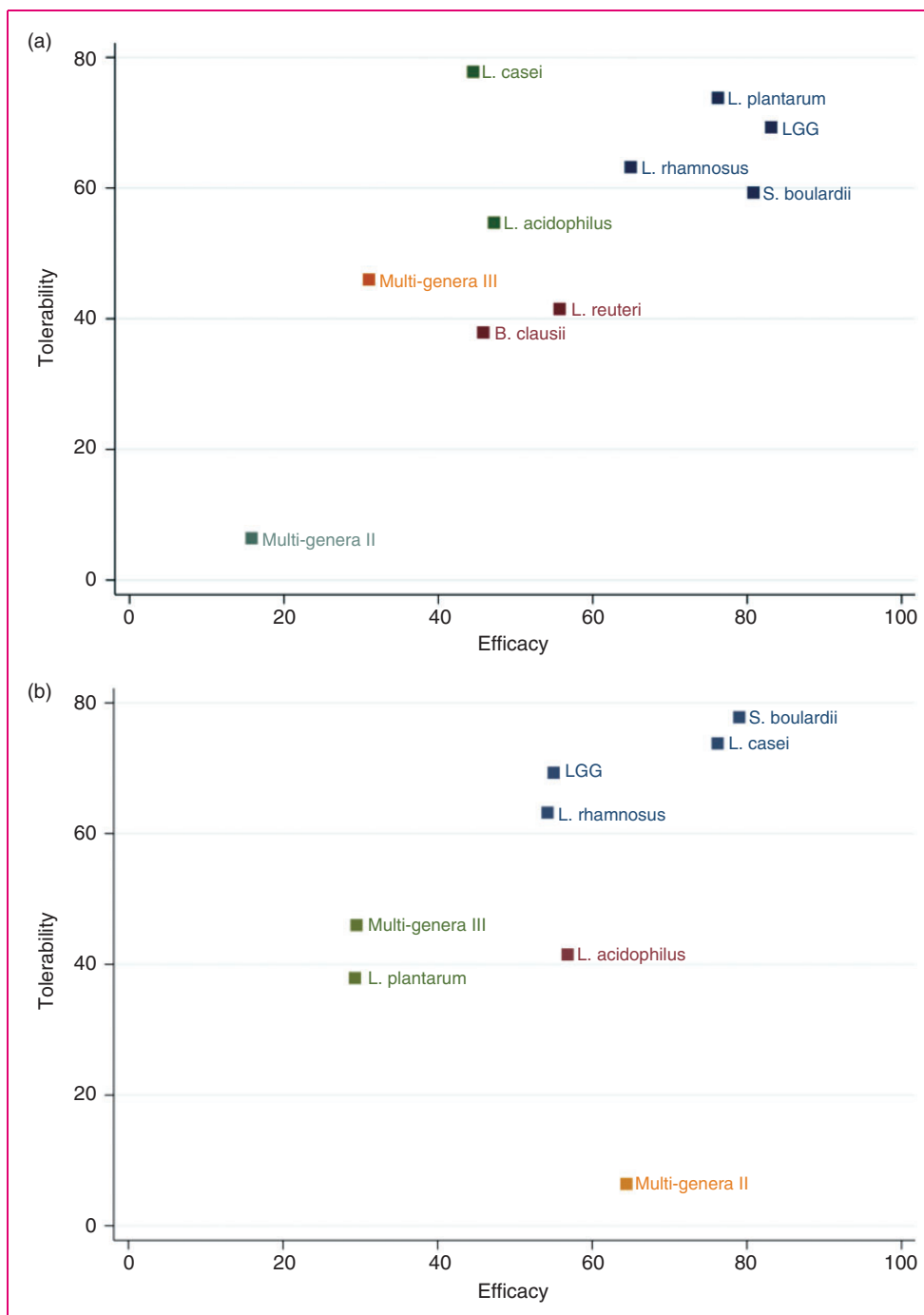
**Figure 4.** Ranking for efficacy and tolerability of probiotic therapies in network meta-analysis. (a) The incidence of diarrhea versus total occurrence of adverse events. (b) *Clostridium difficile* infection rate versus total occurrence of adverse events. LGG: *Lactobacillus rhamnosus* GG.

over single strain alone in either efficacy or tolerability; and (d) no dose correlation of treatment efficacy was indicated based on network model and meta-regression.

Most of our findings shared a similar trend with previous pairwise meta-analyses and clinical trials. A subgroup analysis was conducted by Jafarnejad et al.[33] to estimate comparisons among different genera, which illustrated prevention or treatment of AAD consists primarily of *Lactobacillus* and *S. boulardii*. Moreover, results from Beausoleil and colleagues[34] and Dietrich et al.[2] coincided with the results of *L. casei* in decreasing the incidence of *C. difficile* infection.

Our study was the first network meta-analysis of probiotic preparations for management of AAD. By means of a network approach, we were able to systematically evaluate multiple interventions and to offer a ranking order for each regimen grounded in its capacity to improve clinical efficacy and the probability to cause adverse effects. The integration of indirect and direct evidence leads to a gain of statistical precision compared with previous studies, which also facilitates interpretation because it makes comparisons between therapies explicit.[13,14,33] The design-by-treatment interaction model and node-splitting approach were applied to address concerns regarding potential inconsistency. The GRADE approach was performed to estimate the quality of evidence derived from network and pairwise meta-analysis, which allowed for separate quality assessment for various outcomes. In addition, rigorous screening and large sample size enabled us to provide a precise estimation of major clinical outcomes. Confidence in results is strengthened by the magnitude of effect estimates, stable sensitivity and high consistency between pairwise and network analysis.

The findings of this study might be interpreted considering the following limitations. First, our meta-analysis shares the limitations of the enrolled individual studies. Potential selective reporting, incomplete data, suboptimal allocation concealment and random sequence generation within some individual trials should be considered when interpreting our research. Second, baseline differences in participant-related, intervention-related and disease-related characteristics limited the comparability of trials. To seek heterogeneity, we performed meta-regression to account for the differentials across trials and no major difference was presented, suggesting no significant causes of concern. Moreover, the clinical interpretation of our study is limited owing to the small sample size of some individual studies and the limited number of trials in some nodes. Some of the studies enrolled focused on efficacy outcomes, with limited estimates reported on treatment-related adverse events; hence, we could not perform a thorough assessment of risk-benefit profile in detail. Since trials have been undertaken over 20 years, diagnostic and treatment techniques may have gradually improved. Finally, the extent to which indirect evidence is considered can affect the clinical interpretation of a network meta-analysis. For the incidence of diarrhea, major network inconsistency was not observed in most comparisons, which indicated that results from the indirect estimates were similar to direct evidence. Although node-splitting is limited to closed loops, the most effective or tolerable regimens in our meta-analysis were all part of the closed loops. Confirmation of superiority of these interventions requires direct comparisons, and our rankings should be interpreted modestly as a majority of comparisons across interventions did not obtain statistical significance.

In summary, our network meta-analysis suggests that LGG may be superior to other probiotic treatments for AAD both in efficacy and tolerance. Furthermore, in terms of secondary endpoints, *C. difficile* infection rate, *L. casei* appears to be the most efficacious choice when associated with severe *C. difficile*-related cases. In the future, large, well-designed and multicenter RCTs comparing different probiotic regimens and sensible new options are warranted to achieve a crucial conclusion. More high-quality meta-analyses focused on probiotic dosage, duration and administration timing for different populations should be considered in future studies.

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