



# Efficacy of Single-Strain Probiotics Versus Multi-Strain Mixtures: Systematic Review of Strain and Disease Specificity

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## Abstract

The diversity of probiotic products makes choosing an appropriate probiotic challenging. One unanswered question is whether single-strain probiotics are more effective than multi-strain mixtures. The aim of this review is to account for both disease and strain specificity to determine whether single strains or multiple strains are equivalent or more effective. This literature review of randomized controlled trials from 1973 to 2019 was used to compare the pooled efficacy of trials with a single strain versus the probiotic mixture with same matched strain within the same type of disease indication. A total of 65 RCTs were included (41 with single strains, 22 multi-strain mixtures and 2 comparing single strain to mixture arms) for eight different disease indications ( $N = 10,863$ ). Only three strains (*L. rhamnosus* GG, *L. helveticus* R52 and *B. lactis* Bb12) had corresponding trials with matching mixtures. Use of *L. rhamnosus* GG only was significantly more protective for necrotizing enterocolitis compared to two mixtures also containing different strains of *B. lactis*. The mixture of *L. rhamnosus* GG and *B. lactis* Bb12 was significantly more effective than *L. rhamnosus* GG alone for the eradication of *H. pylori*. In most cases, single strains were equivalent to mixtures. Choice of an appropriate probiotic should be based, not on the number of strains in the product, rather based on evidence-based trials of efficacy. In most cases, multi-strain mixtures were not significantly more effective than single-strain probiotics.

**Keywords** Probiotics · Randomized controlled trials · Strain specificity · Antibiotic-associated diarrhea · Necrotizing enterocolitis · *H. pylori*

## Introduction

Probiotics are a well-studied mode of therapy but not everything is known about how they work and when they are most effective. In addition, regulatory requirements differ by country as probiotics may be available as dietary supplements (not requiring efficacy trials) or as medications (requiring efficacy and safety trials). The choice of when to use specific probiotics is an evolving field. Recent publications have demonstrated both strain and disease specificity for both the prevention and treatment of various diseases [1,

2]. Linking specific probiotic strains based on randomized controlled trials to specific disease indications has become easier due to published practical guidelines, but some recurrent questions are asked by patients and physicians.

One basic question is whether single-strain probiotics or multi-strain probiotic mixtures are better or equivalent for efficacy. Although many single-strain probiotics have evidence-based efficacy, it has been postulated since the intestinal microbiome is complex and contains > 400 species, the use of multiple strains might be more beneficial to restore the microbiome after dysbiosis has occurred. Advantages of multi-strain mixtures may include synergistic effects of different strains in the mixture (increased adhesion, increased pathogen inhibition) [3, 4]. Different strains may also have different mechanisms of actions and thus provide a wider coverage [5, 6]. For example, *B. longum* W11 increases the production of T-helper cells, whereas *B. longum* NCIMB8809 does not [5]. A disadvantage of multi-strain mixtures may be reduced efficacy due to antagonistic intra-strain inhibition by different probiotic strains [7].

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Fredua-Agyeman et al. [8] tested the four individual strains found in a multi-strain mixture (*L. plantarum* 30173, *L. rhamnosus* 30174, *L. acidophilus* 30175, *Enterococcus faecium* 30176, ‘Symprove®’). Two of the four single strains (*L. plantarum* 30173 and *L. rhamnosus* 30174) showed antagonism against the other two strains in the mixture, while no inhibition was found by the other two strains. Another study did not find any intra-strain antagonism between the four strains in a mixture of two lactobacilli strains and two bifidobacterial strains [9].

Two reviews of the literature failed to reach a strong conclusion on this issue citing the lack of studies using the same strains comparing single strain versus multi-strain mixtures as the major reason [10, 11]. Several meta-analyses also attempted to determine whether single strain or multi-strain mixtures are more effective, with most analyses not finding a significant difference, but these meta-analyses pooled different strains of probiotics into either single strain or multiple strain groups and did not account for strain specificity, thus the issue remains unresolved [12–14].

The aim of this paper is to compare the efficacy of single-strained probiotics to corresponding multi-strained mixtures that include at least one identical single strain, while controlling for disease and strain specificities.

## Materials and Methods

As few randomized controlled trials directly compare a single strain to another study arm with a mixture of additional strains, an alternative analytic approach was used. The analysis comprised four steps: (1) literature search of randomized controlled efficacy trials (RCTs) using probiotics, (2) matching of trials within the same disease indication linking single-strain probiotic trials to those with a probiotic mixture containing the identical single strain, (3) pooling outcomes measures from the matched trials using meta-analysis and (4) comparing the pooled efficacy of trials with a single strain versus the probiotic mixture with same matched strain.

### Literature Search

Standard Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines were used for this review (Supplemental Table 1) [15]. PubMed, Google Scholar and NIH registry of clinical trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) were searched from database inception to December 2019, unrestricted by language or year of publication. Search strategy was as follows: (“probiotics” [MeSH Terms] OR “probiotics” [All Fields]) AND “randomized controlled trials” [MeSH Terms] OR “randomized controlled trials” [All Fields] AND “controlled trials”). Additional searches were done using search terms: bacillus or bifidobacterium

or escherichia or enterococcus or lactobacillus or saccharomyces or probiotic mixtures or VSL#3. Secondary searches of grey literature included reference lists, authors, reviews, meeting abstracts websites and [clinicaltrials.gov](http://clinicaltrials.gov) for unpublished trials. A recursive search was also performed, using the bibliographies of all obtained articles. There were no language restrictions. In addition, an open-access database of randomized controlled trials of probiotics was utilized [1] and data were drawn from previously published meta-analyses [16–23].

### Inclusion and Exclusion Criteria

Inclusion criteria included randomized, controlled clinical trials with adult or pediatric patients testing probiotics fulfilling the standard definition (must be living microbe, of adequate dose and having efficacy for a health effect) [24]. As the efficacy of probiotics is disease and strain-specific, as well as mode-specific (prevention or treatment) [25], inclusion of studies into this review was limited to probiotics that had efficacy trials with at least one trial using a single strain and at least one RCT of a multi-strain mixture containing at least one matching single strain. Single-strain probiotics that have not been incorporated into multi-strain mixtures were not included. Multi-strain mixtures that had no corresponding efficacy trials from at least one single strain in the mixture were also excluded. Most trials provided genus and species for probiotics being tested, but if no strain designation could be determined, a search of the product’s website, communication with authors or other manufacturing data were sought to determine the strain designation. As taxonomic classifications of bacteria may shift with updated recommendations, the most current strain designation was assigned to historical taxonomy when appropriate [25]. If no strain designation could be determined, these were excluded. Other exclusion criteria included non-human studies, early phase 1 or 2 safety or mechanism of action studies, no control group, probiotic not well described, reviews and duplicate reports. Cross-over trials were excluded due to the potential for effect carry-over after short wash-out periods used in these trials.

### Statistical Methods

Meta-analyses were conducted separately for each type and mode (prevention or treatment) of disease indication; then, sub-groups of single probiotic strains that had at least one RCT with a sub-group of mixtures containing the matched single strain as one of the strain components were used. Statistical analysis and generation of forest plots of pooled summary estimates was performed using Stata software version 16 (Stata Corporation, College Station, Texas) with meta-analysis modules [26]. Differences in mean daily doses and duration were tested using Student’s *t* test [26]. Publication

bias was assessed using the Egger's test [26]. Heterogeneity was evaluated using  $I^2$  statistic (which indicates the proportion of total variability attributed to heterogeneity) [27]. If significant heterogeneity was found ( $I^2 > 50\%$ ), a random effects model was employed, otherwise fixed-effects models were used. Summary estimates were based on the pooled data from RCTs using the same type (species) of probiotic and sharing a common outcome measure. Dichotomous outcomes were assessed using relative risks (RR) and 95% confidence intervals (CI) and continuous outcomes were assessed using standard mean difference (SMD) and 95% CI using standard methods [15]. Risk of bias was assessed using standard methods, but as inclusion was restricted to RCTs, all included studies had low risk of bias [27].

The efficacy of a single-strain probiotic was compared to matched multi-strained mixtures with the single-strain component and was assessed using tests of interactions from ratios of relative risks (RRR) and 95% confidence intervals (CI) using Z-scores [28] or meta-regression was used for standardized mean differences [26]. Data on patient characteristics, probiotic dose, duration data and individual study quality assessment have been previously published and were not included in this systematic review.

## Results

### Literature Review

A total of 2644 abstracts were initially reviewed (Supplemental Figure 1) and 1751 non-RCT studies were excluded. Of the 893 probiotic trials, 549/893 (62%) used single-strain probiotics and 344 (38%) trials used multi-strained probiotic mixtures. From the literature review, there were 259 different types of probiotic products tested in RCTs, among which 109 were single-strain probiotics. The four most common single-strain probiotics included *Saccharomyces boulardii* CNCM I-745 (109 RCTs), *L. rhamnosus* GG (99 RCTs), *L. reuteri* DSM17938 (22 RCTs) and *Bifidobacterium animalis lactis* Bb12 (18 RCTs). Of the 150 types of multi-strained probiotic mixtures, the three most common mixtures included Lacidofil<sup>®</sup> (*L. helveticus* R52 and *L. rhamnosus* R11, 24 RCTs), VSL#3<sup>®</sup> (*Bifido. breve* DSM24732, *Bifido. longum* DSM24736, *Bifido. infantis* DSM24737, *L. acidophilus* DSM24735, *L. plantarum* DSM24730, *L. paracasei* DSM24733, *L. delbrueckii* subsp. *bulgaricus* DSM24734, *Strept. thermophilus* DSM24731, 20 RCTs) and a two-strain mixture (*L. acidophilus* La5 and *B. lactis* Bb12, 16 RCTs).

However, most multi-strained mixtures (140/150, 93%) did not have trials with a corresponding identical single strain, but ten different multi-strained mixtures had  $\geq 1$  matching RCT with at least one single strain contained in the mixture (a total of 24 RCTs). Some of the common

types of probiotics (*L. reuteri* DSM17938, *L. acidophilus*, *L. plantarum* 299v and *S. boulardii* CNCM I-745) could not be matched with any mixtures that contained the same single-strain type and were excluded. Similarly, the most common multi-strain mixtures did not have corresponding trials with any of the single strains contained in the mixture and were thus excluded. Only three single-strained probiotics (*Bifidobacterium lactis* Bb12, *Lactobacillus rhamnosus* GG, *Lactobacillus helveticus* R52) had RCTs with matching multi-strained mixtures within the same disease indication.

Of the 893 RCTs, 51 of the 59 disease indications could not be included, as there were no matching RCTs with a single strain and mixtures with the corresponding strain within the same disease indication, leaving eight disease indications (total of 81 RCTs). Within the eight disease indications, an additional 16 RCTs were excluded as they did not share common outcome measures with the other RCTs within the same disease indication. For example, the most common outcome measure for IBS was number of 'Responders' (IBS symptom resolution), but one RCT only measured change in quality of life scores and, as a consequence, was excluded. The eight disease indications included treatment of atopic dermatitis/eczema, prevention of atopic dermatitis/allergy, prevention of antibiotic-associated diarrhea (AAD), eradication of *H. pylori*, treatment of irritable bowel syndrome (IBS), prevention of necrotizing enterocolitis (NEC), maintenance of neonatal general wellness and prevention of upper respiratory tract infections (URTI).

A total of 65 RCTs from the eight disease indications were included (41 single-strain trials, 22 multi-strain mixtures and two RCTs comparing the same single strain to a matched multi-strain mixture), including a total  $N = 10,863$  patients [29–93]. The probiotic interventions were found to vary by dose (ranging from  $10^8$  to  $10^{11}$ /day), duration given (ranging from 1 week to 1 year) and country (Supplementary Table 2). Six RCTs provided two different disease indication outcomes (prevention of AAD and eradication of *H. pylori*), as shown in Supplementary Table 3 [34, 35, 37, 39, 41, 44]. Only two studies included a study arm with a single strain directly comparing a matching mixture in another study arm [29, 30].

### Disease-Specific Meta-Analyses

The first step in assessing probiotic efficacy required RCTs to be grouped first by disease indication, then by strain-specific sub-groups with single strains and matching multi-strained mixture subgroups (Supplementary Tables 2–3). The second step compared the pooled estimates (RR or SMD) of single strains to those of the different mixtures (Table 1) to determine whether they were quantitatively different.

**Table 1** Meta-analysis comparing efficacies of single-strain probiotics to multi-strain mixtures within strain types and within disease categories

Disease category	Single strain	Single-strain pooled RR or SMD (95% CI)	Multi-strain mixture	Multi-strain mixtures pooled RR or SMD (95% CI)	Comparative efficacy (RRR or Z test)
Prevention AAD	LrGG	0.48 (0.27, 0.86)	LrGG + BIBb12	0.22 (0.12, 0.39)	2.2 (0.9, 4.9) ns
			LrGG + BIBb12 + LaLa5	0.17 (0.05, 0.64)	2.8 (0.7, 11.4) ns
			LrGG + Lr705 + BbBb99 + Pro-freud	2.09 (0.42, 10.3)	0.2 (0.04, 1.3) ns
Treatment atopic dermatitis	LrGG	0.18 (−0.24, 0.59)	LhR52 + LrR11	0.44 (0.24, 0.79)	0.4 (0.1, 2.1) ns
			LhR52 + LrR11 + B1175 + Sc1079	0.29 (0.03, 2.7)	0.7 (0.05, 9.8) ns
			LrGG + Lr705 + BbBb99 + Pro-freud	0.23 (−0.1, 0.5)	$p=0.94$ ns
Prevention of atopic dermatitis	LrGG	0.84 (0.64, 1.10)	LrGG + Lr705 + BbBb99 + Pro-freud	0.70 (0.51, 0.96)	1.2 (0.8, 1.8) ns
			LrGG + LaLa5 + BIBb12	0.62 (0.46, 0.82)	1.3 (0.9, 2.0) ns
Eradication of <i>H. pylori</i>	LrGG	1.02 (0.90, 1.15)	LrGG + BIBb12	1.20 (1.11, 1.30)	0.8 (0.7, 0.98) <sup>a</sup>
			LrGG + BbBb99 + Profreud	1.15 (0.91, 1.47)	0.9 (0.7, 1.2) ns
			LrGG + Lr705 + Profreud	1.78 (1.19, 2.65)	0.7 (0.3, 1.6) ns
Treatment of IBS	LrGG	1.28 (0.65, 2.54)	LrGG + Lr705 + Profreud	1.78 (1.19, 2.65)	0.7 (0.3, 1.6) ns
Prevention of NEC	BIBb12	0.71 (0.25, 2.03)	BIBb12 + BIBb536	1.84 (0.47, 7.4)	0.4 (0.07, 2.92) ns
			BIBb12 + LrGG	0.78 (0.47, 1.29)	0.2 (0.1, 0.6) <sup>b</sup>
			LrGG + BIBb536	2.18 (0.20, 23.2)	0.1 (0.01, 0.98) <sup>b</sup>
			LrGG + BIHN19	0.51 (0.13, 1.98)	0.3 (0.1, 1.7) ns
Neonatal general health	BIBb12	0.07 (−0.12, 0.25)	BIBb12 + Lr3724	0.44 (0.10, 0.78)	$p=0.2$ ns
Prevention URTI	BIBb12	0.69 (0.53, 0.89)	BIBb12 + LrGG	0.94 (0.79, 1.11)	1.4 (0.99, 1.9) ns
			LrGG	0.75 (0.53, 1.07)	1.2 (0.85, 1.9) ns

AAD antibiotic-associated diarrhea, *B* bifidobacterium, *H. pylori*, *helicobacter pylori*, IBS irritable bowel syndrome, *L. lactobacillus*, ns not significantly different, NEC necrotizing enterocolitis, RR relative risk, RRR ratio of relative risks, SMD standardized mean difference, URTI upper respiratory tract infection, 95% CI 95% confidence interval, Z test Z test for interactions comparing two SMD. Strain designations: BIBb12, *B. animalis* spp. *lactis* Bb12; BbBb99, *B. breve* Bb99 (DSM 13692); BIHN19, *B. lactis* HN19; BIBb536, *B. longum* Bb536; B1175, *B. longum* R175; LaLa5, *L. acidophilus* La5; LhR52, *L. helveticus* R52; Lr705, *L. rhamnosus* LC705 (DSM 7061); LrGG, *L. rhamnosus* GG (ATCC 53103); LrR11, *L. rhamnosus* R11; Lr3724, *L. rhamnosus* CNCM I-3724; Profreud, *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM7076); Sc1079, *S. cerevisiae boulardii* CNCM I-1079

<sup>a</sup> $p < 0.05$  multi-strained mixture better than single strain

<sup>b</sup> $p < 0.05$  single strain better than mixture

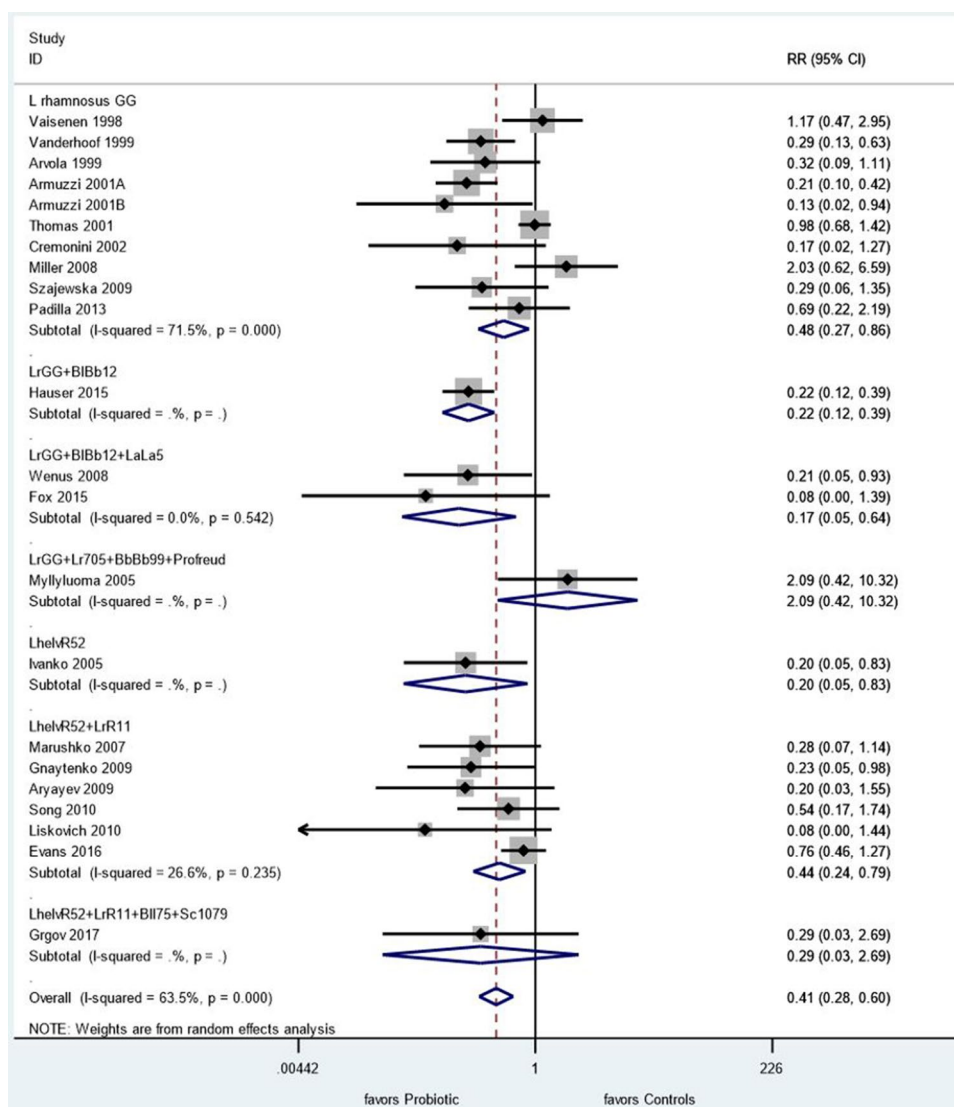
### Prevention of Antibiotic-Associated Diarrhea (AAD)

As shown in Fig. 1 and in Supplementary Table 3, 22 eligible RCTs were found for the prevention of AAD [31–52]. Trials with two single strains (*L. rhamnosus* GG or *L. helveticus* R52) had matching trials using one of the two strains in their mixtures. The pooled estimate from 10 RCTs using *L. rhamnosus* GG alone (RR = 0.48, 95% CI 0.27, 0.86) indicated a significant protective efficacy for the prevention of AAD by this single strain. When another strain (*B. lactis* Bb12) was added, the two-strained mixture was also significantly protective (RR = 0.22, 95% CI 0.12, 0.39). Another mixture containing *L. rhamnosus* GG, *B. lactis* Bb12 and *L. acidophilus* La5 was also found to significantly reduce AAD (RR = 0.17, 95% CI 0.05, 0.64), while a four-strain mixture was not effective (RR = 2.1, 95% CI 0.42, 10.3). The mean daily dose in the ten trials using *L. rhamnosus* GG alone ( $4 \pm 6 \times 10^{10}$ /d) was similar to dose of *L. rhamnosus* GG in mixtures from the four trials ( $9 \pm 8 \times 10^{10}$ /d,  $p = 0.4$ ). The duration of probiotic (1–2 weeks) was also similar for single- and

multi-strained mixtures,  $p = 0.2$  (Supplemental Table 2). Eight RCTs were found using *L. helveticus* R52, with one study finding a significant reduction in AAD when the strain was used by itself (RR = 0.20, 95% CI 0.05, 0.83). Six RCTs with a two-strain mixture of *L. helveticus* R52 and *L. rhamnosus* R11 were also protective (RR = 0.44, 95% CI 0.24, 0.79). However, when two additional strains were added to the mixture (*B. longum* R175 and *S. cerevisiae boulardii* CNCM I-1079), the efficacy was not significant (RR = 0.29, 95% CI 0.03, 2.69). The mean daily dose in the one trial using *L. helveticus* R52 alone ( $2 \times 10^9$ /d) was higher compared to doses of *L. helveticus* R52 when in mixtures in six trials ( $5 \pm 7 \times 10^8$ /d), as only 5% of the mixture was composed of *L. helveticus* R52. The duration of probiotic (1–4 weeks) was also similar for single- and multi-strained mixtures (Supplemental Table 2). As shown in Table 1, none of the five mixtures were significantly more effective than use of the single probiotic strains (*L. rhamnosus* GG or *L. helveticus* R52) used alone. There was no significant publication bias (Egger  $p = 0.23$ ).



**Fig. 1** Forest plot of 22 probiotic randomized controlled trials for prevention of antibiotic-associated diarrhea. *B. bifidobacterium*, *L. lactobacillus*, RR relative risk, 95% CI 95% confidence interval. Strain designations: BIBb12, *B. animalis* spp. *lactis* Bb12; BbBb99, *B. breve* Bb99 (DSM 13692); B1175, *B. longum* R175; *L. helveticus* R52; LrGG, *L. rhamnosus* GG (ATCC 53103); LrR11, *L. rhamnosus* R11; Profreud, *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM7076); Sc1079, *S. cerevisiae* CNCM I-1079



### Treatment of Atopic Dermatitis/Eczema

Seven RCTs were found using *L. rhamnosus* GG alone or as a mixture (*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and *Propionibacterium freudenreichii shermanii* JC) [29, 53–57]. The mean daily dose in the six trials using *L. rhamnosus* GG alone ( $2 \pm 1 \times 10^{10}/d$ ) was similar to the one treatment arm that included *L. rhamnosus* GG in the mixture ( $1 \times 10^{10}/d$ ). Neither the duration of probiotic given nor the country was significantly different for single versus mixtures (Supplemental Table 2). Pooled outcome data (Supplemental Figure 2) found neither the use of the single *L. rhamnosus* GG strain (RR = 0.18, 95% CI – 0.24, 0.59) nor the four-strain mixture (RR = 0.23, 95% CI – 0.09, 0.55) was significantly effective for the treatment of atopic dermatitis ( $t = -0.08$ ,  $p = 0.94$  from meta-regression models). There was no significant publication bias (Egger's  $p = 0.79$ ).

### Prevention of Atopic Dermatitis/Allergy

Seven RCTs were found using *L. rhamnosus* GG alone (4 RCTs) or included into a multi-strain mixture (3 RCTs) with varying results (Supplementary Table 3) [58–64]. The mean daily dose in the four trials using *L. rhamnosus* GG alone was similar ( $2 \pm 0.5 \times 10^{10}/d$ ,  $p = 0.1$ ) to the mean dose used in three trials that included *L. rhamnosus* GG in their mixture ( $4 \pm 2 \times 10^{10}/d$ ). The duration of probiotic (mean 4 months) was similar in both single- and multi-strained mixtures (Supplemental Table 2). Meta-analysis (Supplemental Figure 3) found *L. rhamnosus* GG by itself did not significantly reduce atopic dermatitis or pollen allergy (RR = 0.90, 95% CI 0.58, 1.39). Two mixtures were able to significantly reduce the incidence of atopic dermatitis: *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99, *Prop. Freudenreichii shermanii* JC (RR = 0.70, 95% CI 0.51, 0.96) and *L. rhamnosus* GG, *L. acidophilus* La5 and *B. lactis* Bb12

(RR = 0.62, 95% CI 0.46, 0.82). However, neither mixtures were significantly more protective compared to the single probiotic strain (Table 1). There was no significant publication bias (Egger  $p = 0.14$ ).

### Eradication of *H. pylori*

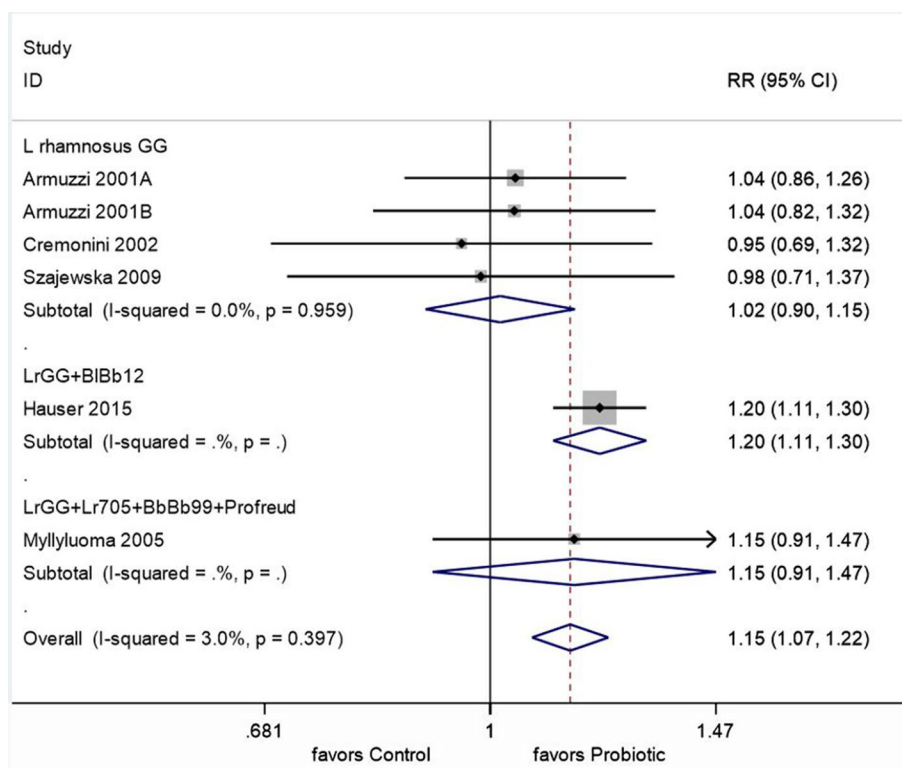
Probiotics have been added to standard eradication treatments in an effort to either increase compliance (which may indirectly improve cure rates) or as a direct mechanism to clear the pathogen, as some probiotics interfere with the attachment of *H. pylori* [19, 20]. Six RCTs were found using *L. rhamnosus* alone (4 RCT) or a two-strain mixture (*L. rhamnosus* GG, *B. lactis* Bb12) and a four-strain mixture (*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and *Prop. Freudenreichii shermanii* JC) [34, 35, 37, 39, 41, 44]. No significant efficacy was found for the single strain alone (RR = 1.02, 95% CI 0.90, 1.15) or the four-strain mixture (RR = 1.15, 95% CI 0.91, 1.47), but the two-strain mixture had a significant cure rate for *H. pylori* (RR = 1.20, 95% CI 1.11, 1.30), as shown in Fig. 2. The mean daily dose in the four trials using *L. rhamnosus* GG alone ( $7 \pm 4 \times 10^9$ /d) was similar to the dose of *L. rhamnosus* GG in mixtures in two trials ( $4 \pm 4 \times 10^9$ /d,  $p = 0.5$ ). The duration of probiotic (1–4 weeks) was also similar for single- and multi-strained mixtures (Supplemental Table 2). The mixture containing *L. rhamnosus* GG

and *B. lactis* Bb12 was significantly better than the single strain of *L. rhamnosus* GG alone (RRR = 0.85, 95% CI 0.73, 0.98). There was significant publication bias found for this indication (Egger  $p = 0.01$ ).

### Treatment of IBS

Four RCTs were found using *L. rhamnosus* GG alone (3 RCTs) and one trial with four strains (*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and *Prop. Freudenreichii shermanii* JC) [65–68]. The mean daily dose in the three trials using *L. rhamnosus* GG alone ( $9 \pm 1 \times 10^9$ /d) was similar to the one trial that included *L. rhamnosus* GG in their mixture ( $9 \times 10^9$ /d). The duration of probiotic (4–8 weeks) was shorter for the three single-strain trials compared to the one multi-strained mixture (duration 24 weeks), as shown in Supplemental Table 2. A meta-analysis (Supplemental Figure 4) found the single strain of *L. rhamnosus* GG did not significantly increase the responder rate for IBS (RR = 1.28, 95% CI 0.65, 2.54), but the four-strain mixture did significantly increase the responder rate (RR = 1.78, 95% CI 1.19, 2.65). However, the mixture was not significantly increased over the single strain (RRR = 0.72, 95% CI 0.32, 1.58), perhaps due to the low number of RCTs using the mixture (only one trial). There was no significant publication bias (Egger  $p = 0.37$ ).

**Fig. 2** Forest plot of probiotic randomized controlled trials for eradication of *H. pylori*. Outcome is eradication rate. Strain designations of mixtures: B1Bb12, *B. animalis* spp. *lactis* Bb12; BbBb99, *B. breve* Bb99 (DSM 13692); Lr705, *L. rhamnosus* LC705 (DSM 7061); LrGG, *L. rhamnosus* GG (ATCC 53103); Profreud, *Propionibacterium freudenreichii* ssp. *shermanii* JS



## Prevention of NEC

Ten RCTs were found using either *B. lactis* Bb12 alone (3 RCTs) or *L. rhamnosus* GG alone (4 RCTs) and four two-strain mixtures (1 RCT each): *B. lactis* Bb12 and *B. longum* Bb536 or *L. rhamnosus* GG and *B. lactis* Bb12 or *L. rhamnosus* GG and *B. lactis* Bb536 or *L. rhamnosus* GG and *B. lactis* HN19 [30, 69–77]. The mean daily dose in the three trials using *B. lactis* Bb12 alone ( $5.3 \pm 4.5 \times 10^9/\text{d}$ ,  $p=0.4$ ) was similar to the two trials ( $2 \times 10^9/\text{day}$ ) when in mixtures. In contrast, the mean dose in the four trials using *L. rhamnosus* GG alone was significantly higher ( $6 \pm 0 \times 10^9/\text{d}$ ) than the mean dose of *L. rhamnosus* GG when used in mixtures ( $3 \pm 1 \times 10^8/\text{d}$ ,  $p < 0.001$ ). The duration of probiotic (mean of  $5 \pm 1$  weeks for both groups) was also similar for single- and multi-strained mixtures (Supplemental Table 2). A meta-analysis (Supplemental Figure 5) found the only significant efficacy was found for *L. rhamnosus* GG alone (RR = 0.17, 95% CI 0.07, 0.41). *L. rhamnosus* GG alone was also significantly more effective than the mixture of *L. rhamnosus* GG and *B. lactis* Bb12 (RRR = 0.22, 95% CI 0.08, 0.60) and the mixture of *L. rhamnosus* GG and *B. longum* Bb536 (RRR = 0.08, 95% CI 0.01, 0.98), as shown in Table 1. There was no significant publication bias (Egger  $p=0.86$ ).

## Maintenance of Neonatal General Health

Six RCTs were found for assessing use of probiotics for neonatal health with the common outcome of increase in body weight while on probiotics [78–83]. Most were done using *B. lactis* Bb12 alone (5 RCTs) and one trial used a mixture of *B. lactis* Bb12 with *L. rhamnosus* CNCM I-3724. The mean daily dose in the five trials using *B. lactis* Bb12 alone ( $1 \pm 2 \times 10^9/\text{d}$ ) was similar to the one trial that included *B. lactis* Bb12 in the mixture ( $1 \times 10^9/\text{d}$ ). The duration of probiotic for single strains varied from 4–12 months, while the mixture was only given for 2 months (Supplemental Table 2). While the single strain by itself did not result in a significant gain in body weight, the mixture resulted in mild increase (RR = 0.44, 95% CI 0.10, 0.78), (Supplemental Figure 6). The mixture was not significantly better than the single strain alone ( $t = 1.86$ ,  $p = 0.20$  from meta-regression models), as shown in Table 1. There was no significant publication bias (Egger  $p = 0.63$ ).

## Prevention of URTI

Ten RCTs were found for the prevention of respiratory tract infections, either as a single strain (*B. lactis* Bb12, 1 RCT or *L. rhamnosus* GG, 6 RCTs) or a mixture of the two strains (3 RCTs) [84–93]. The mean daily dose in the six trials using *L. rhamnosus* GG alone ( $2 \pm 4 \times 10^9/\text{d}$ ,  $p = 0.3$ ) or the one trial using *B. lactis* Bb12 alone ( $1 \times 10^9/\text{d}$ ) was similar to

the three trials that included *L. rhamnosus* GG or *B. lactis* Bb12 in their mixture ( $3 \pm 3 \times 10^9/\text{d}$  each strain). The duration of probiotic varied widely, with single strains given for a shorter mean duration ( $11 \pm 12$  weeks) were compared to  $26.4 \pm 19.2$  weeks for the mixtures, but the difference was not significant ( $p = 0.2$ ). Only *B. lactis* Bb12 alone showed significant efficacy (RR = 0.69, 95% CI 0.53, 0.89), while *L. rhamnosus* GG alone did not (RR = 0.75, 95% CI 0.53, 1.07) (Supplemental Figure 7). The combination of the two strains together did not show an increase in efficacy (Table 1) despite being given for longer times. The mixture was not significantly different from either single strain alone: *B. lactis* Bb12 (RRR = 1.36, 95% CI 0.99, 1.86) or *L. rhamnosus* GG alone (RRR = 1.25, 95% CI 0.85, 1.85). There was no significant publication bias (Egger  $p = 0.69$ ).

## Discussion

This study demonstrated, at least for the limited number of strains that could be evaluated, when disease and strain specificity is accounted for, the efficacy of probiotics is typically not more effective for multiple strains compared to single strains. Only two trials directly compared a single-strain probiotic group to a study arm using a mixture with additional strains, but neither study found a significant difference between the single strain and the mixtures [29, 30]. Lacking more direct comparison trials, pooling trials within the same strain types was productive. A single strain (*L. rhamnosus* GG) was found more effective than two mixtures containing additional strains for the prevention of necrotizing enterocolitis. A two-strain mixture of *L. rhamnosus* GG and *B. lactis* Bb12 was more effective than either strain alone for the eradication of *H. pylori* when used as an adjunct to antibiotic treatments. In all other comparisons, there was no increased efficacy found when additional probiotic strains were added to an effective single probiotic strain.

With recent publications demonstrating strain and disease specificity observed in clinical trials of efficacy [1], the importance of only comparing the same strains to each other has become apparent. Other studies concluding multi-strained probiotic mixtures were more effective than single-strained probiotics based on their conclusions on biased data, in that the group of multi-strained probiotics was often different types of mixtures. Chang et al. [14] reported ‘multiple strain probiotics’ appeared to be more effective than single strains for the prevention of NEC and mortality, but of the 11 trials pooled into one group of ‘multi-strained’ probiotics, there were actually 10 different types of mixtures of varying efficacies. Several other studies have combined different types of mixtures and pooled different types of single strains into groups and either concluded there was no significant difference between single- and multi-strain

probiotics [12, 13, 94]. Chapman et al. [95] reviewed probiotic literature and reported mixtures were more effective in 12/16 (75%) of the studies (including in vitro studies, animal models and RCTs). However, only two studies were RCTs with clinical outcomes and neither study defined the strain(s) of probiotics used. Ouwehand et al. [11] reviewed over 60 RCTs and compared all single-strained probiotics against multi-strain mixtures within 39 different disease indications. Both this extensive review [11] and a short review [10] were unable to come to a conclusion due to the paucity of trials with matching single strain and multi-strain mixtures. None of these reviews quantitatively compared single probiotic strains with matched multi-strain mixtures.

The different efficacies of probiotic strains may be due to varying mechanisms of actions possessed by different strains and if they are given singly or in combination with other strains. A clear advantage of a single strain was only found for *Lactobacillus rhamnosus* GG (ATCC 53103) for the prevention of necrotizing enterocolitis. Mileti et al. [6] studied three strains of lactobacilli (including *L. rhamnosus* GG) and reported differing abilities to produce cytokines by strain. In our study, addition of *B. lactis* Bb12 to *L. rhamnosus* GG did not result in a synergistic effect for seven of the different disease indications. In only one case was the mixture of *L. rhamnosus* GG and *B. lactis* Bb12 more effective than either of the single strains given alone (for the eradication of *H. pylori*). A basis for this synergistic effect may be due to the doubling of the adhesion of *B. lactis* Bb12 when combined with *L. rhamnosus* GG [3]. Few strains had evidence from trials for a single strain linked with trials of additional strains. However, in the case of *L. helveticus* R52, the single strain significantly prevented AAD, but the addition of another strain (*L. rhamnosus* R11) did not significantly improve this efficacy, which might reinforce the efficacy to the R52 strain. Interestingly, when two additional strains were added (*B. longum* I-175 and *S. cerevisiae* I-1079), this 4-strain mixture was no longer significantly protective of AAD. An explanation for this lack of protective effect might be the observed reduction in cytokine production in this four-strain mixture compared to cytokine levels when the single strains were tested independently [96].

There are several strengths of this review. This is the first study directly linking the efficacy of single-strained probiotics to a multi-strain mixture containing the same strain and comparing efficacy within the same type and mode (prevention or treatment) of disease. Unlike previous reviews that qualitatively compared the efficacy of single strain and multi-strain mixtures, we used quantitative tests to determine whether there is a significant difference between these types of probiotics. We extensively reviewed a large number of trials ( $n = 65$  RCTs) over eight different types of diseases.

There are several limitations of this review. Although the research on probiotics has been done in a variety of disease

indications when determining whether singlestrains or multiple strains are more effective, the lack of RCTs using the same strains for single strain versus multi-strain mixtures limited the types of probiotics we could analyze. Many of the most commonly used probiotics could not be included in this analysis due to a lack of trials using the same single strain and trials with the identical strain in multi-strain mixtures. For example, one of the more commonly researched probiotic, *S. boulardii* CNCM I-745, could not be assessed, as all mixtures either used another strain (*S. cerevisiae* CNCM I-1079) or did not provide the strain of *Saccharomyces* used. Another limitation was the lack of strain designations in many single strain or multi-strain mixtures. In addition, not all strains used in multi-strained mixtures had corresponding trials with each individual strain of probiotic. Another limitation was the lack of common outcome measures used for the same type of disease (most notable, IBS outcomes ranged from change in symptom scores, to quality of life measures or frequency of abdominal pain/gas). In most cases, the potential for synergistic or antagonistic effects in multi-strained mixtures could not be evaluated, as most single strains in mixtures have not been tested independently. The variety of daily doses and duration of probiotics given varied, even within disease indications and, in general, daily doses or duration were not significantly different. In one case, the significant efficacy for the single strain of *L. rhamnosus* GG compared to mixtures in the prevention of NEC might have been due to the significantly higher dose used in the single-strain trials. Other confounding factors, such as differences in dietary patterns in different countries, study population characteristics (age, race, etc.) were not analyzed in this review.

The choice of an appropriate probiotic product for patients continues to be a clinical challenge, and as not all probiotics are equally effective or cost-effective, the decision must be based on available scientific evidence. Future trials should compare similar doses and durations of single strains of probiotics with multi-strain mixtures containing the identical single strain within the same disease indication. Adding a previously untested probiotic strain to a mixture should carefully consider interactions between the strains, doses and durations given and how an increase in efficacy is documented. Each single strain should be tested independently with clearly defined parameters (study population, daily dose and duration of probiotic given, disease outcome measures) and then a mixture made from the previously studied single strains (at the same dose) can be tested for any increased efficacy. In addition, other important considerations, such as cost-effectiveness, frequency of side effects and impact on the normal microbiome need exploration, as this information was not found in most of the included trials in this review. More studies comparing the same strains given singly versus given in a mixture within the same disease indication



are needed to clearly determine whether single strains or mixtures are more effective. This study shows that, in most cases, it is not just the number of strains used to prevent or treat specific diseases that predicts efficacy, rather it is the choice of which strain that is the most important factor impacting efficacy.

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## Compliance with Ethical Standards

**Conflict of interest** LVM is on the Scientific Advisory Board and paid consultant for Bio-K+ (Canada) and on the Microbiome Advisory Board and paid consultant for Biocodex (France) but owns no stock or equity in either company.

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