



**A systematic review for evidence-based studies of probiotics supporting a health claim for microbial dysbiosis.**

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## A systematic review for evidence-based studies of probiotics supporting a health claim for microbial dysbiosis.

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## ABSTRACT

**Objective:** To quantify and analyse the quality of evidence for the claim that probiotics can correct dysbiosis of the normal microbiota from randomised controlled trials.

**Setting:** Systematic review of current published clinical trials of adult or pediatric patients receiving a probiotic intervention for the prevention or treatment of various diseases.

**Data sources:** Sources searched: PubMed (1985-2013), EMBASE (1985-2013), Cochrane Database of Systematic Reviews (1990-2013), CINAHL (1985-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.

**Review methods:** Included trials were randomised evaluations of host normal microbiota embedded in trials of probiotic interventions. Studies were evaluated following PRISMA guidelines for specific probiotic strains. A standard data extraction form was used to collect the raw data. The degree of dysbiosis correction is grouped into three different outcomes (restoration, alteration or no effect) depending upon the type of study design used (Models A-C).

**Outcome measures:** The primary outcome is the degree of microbiota correction by specific probiotic strains. Secondary outcome was the association of the degree of dysbiosis correction and the strength of efficacy found in randomized controlled trials.

**Results:** 63 trials (with 69 treatment arms) are included. Complete restoration of the microbiota was found in 83% of 12 probiotic products, altered microbiota was documented in 56% of 18 probiotics and no change in microbiota was found in 79% of 19 probiotics. Clinical efficacy was associated with strains capable of restoration of the normal microbiota.

**Conclusions:** Only five (10%) of the 49 probiotic strains have evidence for normal microbiota restoration or alteration with supportive clinical efficacy trial results. The health claim for correcting dysbiosis is poorly supported for most probiotic strains and requires further research.

**Systematic review registration:** PROSPERO (CRD42014007224)

## Strengths and Limitations

### Strengths include:

- A comprehensive review of the published literature from 1985-2013
- Literature search unrestricted by language or country
- Analysis of study designs resulted in novel strategy to limit bias and classify outcomes
- Three types of outcomes of dysbiosis applied to evidence-based studies of specific probiotic strains
- Author has over 40 years of research experience in the probiotic field

### Limitations include:

- Pooled clinical trials using different study populations
- Pooled probiotic doses and regimens
- Indirect evidence linking probiotic strains and dysbiosis
- Review done by sole author

## INTRODUCTION

The popularity of probiotics has expanded exponentially recently, but along with their increased use, debate rages on how probiotics should be regulated and whether probiotics should be considered as a drug or a food supplement. In the U.S., unlike approved prescription drugs, which are regulated by the Food and Drug Administration (FDA), probiotics are typically available as over-the-counter medications or as dietary supplements and thus are limited to 'structure or function health claims' and are not permitted to claim to 'treat' or 'cure' disease. In Europe and the United Kingdom, probiotics are allowed to have 'disease or symptom claims'. These claims are required to be supported by well-conducted human trials in the targeted population or in healthy volunteers, but the European Food Safety Authority (EFSA) has rejected 80% of claims submitted to them.<sup>1-3</sup> In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect.<sup>4</sup> One such health claim made for probiotic products is that they correct dysbiosis (or the disruption of bacterial and fungal species after antibiotics or other disruptive exposures) and thus may be beneficial to maintain health. Probiotics are uniquely qualified act as temporary surrogate normal microbiota or act to protect the disrupted niche until dysbiosis is corrected. A wide variety of mechanisms-of-action have been documented for probiotics (ranging from blocking pathogen attachment sites, destruction of the pathogen by bacteriocins or proteases that degrade toxins to regulation of the immune system),<sup>5,6</sup> and while clinical evidence supports efficacy of some probiotic strains, the evidence linking these mechanisms-of-action to health claims is not as clear.

A classic example of the consequence of dysbiosis is antibiotic-associated diarrhea (AAD).<sup>7,8</sup> While antibiotics may be effective in the elimination of pathogenic organisms, a common, unintended effect is the killing or inhibition of beneficial microbes due to shared susceptibility to the antibiotic. One of the many functions for normal microbiota is the ability to resist infection by pathogenic organisms, termed 'colonization resistance'.<sup>9,10</sup> The loss of a sub-population of the normal microbiota, for example, can lead to the loss of the ability to break down fibers and starches into absorbable short chain fatty acids, resulting in high level of undigested carbohydrates, which can trigger diarrhea.<sup>11</sup> Disruption of the normal microbiota has been

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3 shown to lead to higher rates of infections in other body systems other than the intestinal tract  
4 including the skin,<sup>12,13</sup> vagina,<sup>14,15</sup> respiratory tract,<sup>16,17</sup> and in the buccal cavity.<sup>18-20</sup>  
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10 The challenge to proving the microbiota has been restored after a disrupting event is that it is  
11 comprised of  $>10^{13}$ - $10^{14}$  organisms and standard microbial culturing methods miss 75-95% of  
12 these organisms.<sup>21,22</sup> The development of metagenomics (cataloguing individual and disease-  
13 specific bacterial gene profiles) and the creation of the international Human Microbiome Project  
14 ushered in a new era for our understanding of the complexity of these interactions within the  
15 body.<sup>23,24</sup> This paradigm shift from culturing to metagenomic analysis has expanded our ability  
16 to document shifts in microbial populations to an unparalleled degree, but the interpretation of  
17 these shifts continues to be under debate.<sup>25-28</sup> With the advent of these newer metagenomic  
18 tools, the role of probiotics in the restoration of normal microbiota is being re-visited.<sup>29</sup>  
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30 In light of new guidance documents and recommendations, the goal of this systematic review is  
31 to determine if health claims for the restoration of the normal microbiota and the correction of  
32 dysbiosis have been studied with well-designed trials and which probiotic strains have evidence-  
33 based data to support these claims.  
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## 39 **METHODS**

### 40 **Study Objective**

41 To systematically review the literature to analyse the quality of evidence for the claim that  
42 probiotics can correct dysbiosis of the normal microbiota from randomised controlled trials.  
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### 49 **Search Strategy**

50 Search terms included: health claims for restoring normal microbiota, dysbiosis, normal  
51 microbiota, pharmacokinetics, metagenomics, probiotics, dietary supplements, randomized  
52 controlled trials and specific probiotic strains or products. Search strategies were broad-based  
53 initially, then narrowed to clinical trials with probiotics.  
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## Data Sources

PubMed (1985-2013), EMBASE (1985-2013), Cochrane Database of Systematic Reviews (1990-2013), CINAHL (1985-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials (<http://www.cochrane.org>), MetaRegister of Controlled Trials (<http://www.controlled-trials.com/mrct>) and National Institutes of Health (<http://www.clinicaltrials.gov>).

## Criteria for study selection and data extraction

Abstracts of all citations were reviewed and rated for inclusion for randomized controlled trials of probiotic treatments. Full articles were retrieved if normal microbiota assays were mentioned. Non-English language trials were translated and included whenever possible. Exclusion criteria included pre-clinical studies (animal models or *in vitro* assays), safety or phase 2 studies, reviews, efficacy trials with no assays for normal microbiota species, metagenomic methods only, mechanism of action of normal microbiota or probiotic, cross-sectional surveys, case reports or case series, duplicate reports, or trials of unspecified types of probiotics. All pharmacokinetic studies in humans were reviewed, as abstracts often did not include normal microbiota assay data. Data extraction and the review process followed the PRISMA statement guidelines using a 27-item checklist and flow diagram.<sup>30</sup> A standardized data extraction form was used to collect data on the probiotic (strain type, daily dose, duration), type of controls (placebo, active or no treatment), study design (status of microbiota at baseline and follow-up times), type of microbiota assay (microbial culturing, molecular biomarkers, etc.), enrolled study population (adult vs. pediatric, healthy volunteers, disease condition), type and timing of disruptive agent (antibiotics, chemotherapy, etc.), study size and attrition, outcome assessment (efficacy and/or microbiota status at end of study, adverse events) and type of health claim.

## Outcomes and definitions

The primary outcome is the degree of microbiota correction or improvement by the probiotic strain. The secondary outcome is the association of the degree of dysbiosis correction and the strength of efficacy found in randomized controlled trials of probiotic interventions. Dysbiosis is defined as an alteration or disruption of the normal microbiota (bacterial or fungal species) due

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3 to exposure of an inciting factor (such as antibiotics, chronic disease, stress, medical procedures  
4 or medications, etc.). Included studies were required to have at least a pre-probiotic treatment  
5 assay and a post-probiotic treatment assay for normal microbiota determination. As a variety of  
6 microbial assays were available during the search period (1985-2013), we included  
7 documentation of the microbiota by either microbial cultures, or metagenomic methods [16s  
8 rRNA-targeted probes using fluorescent *in situ* hybridization (FISH) or other polymerase chain  
9 reaction (PCR) technique]<sup>8,21,28,31</sup> or by indirect methods (Nugent scores).<sup>15</sup> Nugent scores  
10 (ranged 0-10) are used to diagnose bacterial vaginosis (scores  $\geq 7$ ) or normal vaginal microbiota  
11 (scores 0-3) based on the quantitated morphotypes of small gram negative rods (*G.*  
12 *vaginalis*/*Bacteroides* spp.) and curved gram negative rods (*Mobiluncus* spp.) from gram stains  
13 of vaginal discharge smears. Microbial assays of the strain(s) contained in the probiotic product  
14 are considered as pharmacokinetic studies and were not included in the normal microbiota  
15 profiles.  
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28 To determine the impact on normal microbiota, only direct evidence of microbiota change  
29 (species, profiles, diversity indices, or diagnostic criteria) were included and indirect effects were  
30 excluded (changes in intestinal enzymes, short chain fatty acids, immune system parameters or  
31 disease symptoms). The degree to which dysbiosis was improved is categorized into three  
32 levels: (1) recovery of the normal microbiota back to baseline levels; (2) alteration or  
33 improvement of the normal microbiota; and (3) no change in normal microbiota. 'Recovery' of  
34 the normal microbiota is defined as a restoration of the microbiota back to a normal baseline. To  
35 determine this outcome, only those studies that enrolled subjects with undisrupted microflora at  
36 baseline and assayed subjects before a disruptive event occurred (such as antibiotic exposure or  
37 chronic disease) and compared the microbiota using another assay taken after the study  
38 intervention was completed were used, as shown in Figure 1. These types of clinical types are  
39 typically preventive probiotic interventions. This type of study design is termed 'Model A'.  
40 Recovery may be complete recovery (all assayed microbial levels returned to baseline) or  
41 incomplete recovery (partial recovery of some microbial strains, but not all returned to baseline  
42 levels). In studies enrolling subjects with dysbiosis at baseline (typically due to chronic  
43 diseases), it is not possible to show a restoration to normal microbiota levels because a normal,  
44 undisturbed microbiota was not present in these types of study subjects at the time of enrollment.  
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3 Therefore, the strongest claim possible is for an 'alteration or improvement' of the normal  
4 microbiota. To determine this outcome, studies in which the first assay occurred after a  
5 disruptive exposure (e.g., post-antibiotic exposure or during active disease) and the second assay  
6 occurred post-probiotic intervention were grouped (Model B in Figure 1). These types of studies  
7 are typically for the treatment of existing diseases by probiotics. In studies enrolling healthy  
8 subjects who had a normal microbiota at baseline and were not exposed to a disruptive factor at  
9 any time during the study intervention trial, dysbiosis did not occur. These types of clinical trials  
10 enrolled healthy volunteers and were assayed before and after the probiotic intervention was  
11 given (Model C in Figure 1). Only data from the probiotic-exposed subjects were analysed in this  
12 paper. Data from the control groups were used to confirm dysbiosis for subjects with chronic  
13 diseases or after a disruptive exposure, such as antibiotics or chemotherapy, unaffected by  
14 probiotic exposure.<sup>32-34</sup>

### 25 26 **Assessment of methodological quality**

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28 The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system  
29 for rating overall study quality will be used for each probiotic strain or type (single strains and  
30 mixtures of strains).<sup>35</sup> Recommendation for the support of the claim of each probiotic strain or  
31 mixture can be assessed by the overall strength of the evidence [“strong”, many randomized  
32 controlled trials show significant recovery of the microbiota, or “moderate” only one randomized  
33 controlled trial; or “weak”, only case series or reports, limited number of small trials, *etc.*].  
34 Quality of the evidence is graded as “high quality” (well-defined study design for determining  
35 restoration with normal microbiota at baseline), or “moderate quality” (disrupted microbiota at  
36 baseline), or “low quality” (no disruptive event occurred). Measurement of publication bias was  
37 not assessed for this review, as pooled outcome estimates of efficacy were not done, as typical in  
38 meta-analysis, but all studies with assays of microbiota were included to limit bias.

### 39 40 41 **Net efficacy rating**

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43 To determine if the ability to correct dysbiosis is associated with clinical efficacy, we reviewed  
44 the published literature for randomized controlled trials (RCTs) or meta-analyses of probiotics  
45 for various disease indications, including antibiotic associated diarrhea (AAD),<sup>5,36,37</sup> *Clostridium*  
46 *difficile* infection (CDI),<sup>5,38</sup> inflammatory bowel disease (IBD),<sup>39</sup> irritable bowel syndrome

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3 (IBS),<sup>40</sup> traveler's diarrhea (TD),<sup>41</sup> eradication of *Helicobacter pylori* (Hp),<sup>36,37</sup> bacterial  
4 vaginosis (BV) or vaginitis,<sup>42</sup> and treatment of acute pediatric diarrhea.<sup>43-45</sup> The net rank was  
5 calculated by subtracting the number of RCTs showing non-significant or equivalent efficacy  
6 from the number of RCTs having significant efficacies. The ranks were categorized as follows:  
7 ++,  $\geq 2$  net RCTs showing significant efficacy; +, net of one RCT showing significant efficacy; 0,  
8 equal number of RCTs showing significant and non-significant efficacy results and -,  $\geq 1$   
9 negative or non-significant RCTs. Probiotics with no RCTs were not ranked.  
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## 18 RESULTS

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20 A review of the literature from 1985-2013 found 353 articles that dealt with probiotic treatments  
21 and their potential effect on normal microbiota.  
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### 26 Excluded studies

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28 As shown in Figure 2, a total of 272 articles were excluded for the following reasons: reviews  
29 (n=116), probiotic efficacy studies with no data on normal microbiota assays (n=54), animal  
30 models of probiotics and changes in microbiota (n=38), metagenomic or microbiota methods  
31 only (n=17), studies on normal microbiota but with no use of probiotics (n=14), *in vitro* assays of  
32 microbiota (n=10), or miscellaneous (n=23), which included probiotic mechanism of action  
33 studies, safety studies, duplicative reports, cross-sectional surveys and two with poorly described  
34 probiotic interventions.<sup>46,47</sup> We reviewed 81 full articles which mentioned changes in normal  
35 microbiota or indicated a health claim for probiotics and effects on normal microbiota.  
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44 **Probiotic pharmacokinetic studies.** . We found 18 studies reporting concentrations of probiotic  
45 strains before and post-treatment, but did not assay for other species of normal microbiota.  
46 Studies that assay for only the probiotic strain(s) that are given, and do not assay for other  
47 normal microbiota bacteria or fungi, cannot determine what impact the probiotics have on  
48 normal microbiota. While several studies using this study design claim probiotics had an impact  
49 on normal microbiota, type of data generated is pharmacokinetic behavior of the probiotics  
50 themselves and not the normal microbiota. Several studies stated that the normal microbiota was  
51 altered because an increase in various bacterial species was observed after the probiotics were  
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3 given, but the species assayed were those contained in the probiotic product, so an increase is not  
4 unexpected. Pharmacokinetic studies have documented that probiotic strains taken orally can  
5 survive transit through the intestinal tract with recovery rates in feces ranging from <1% to  
6 22%.<sup>48,49</sup> These pharmacokinetic studies were excluded from this analysis, as they did not assay  
7 other types of normal microbiota not found in the probiotic product.  
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### 13 14 **Included studies**

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16 Of the 63 included clinical trials, five trials had multiple treatment arms, which resulted in a total  
17 of 68 treatment arms for analysis. Engelbrekton et al. tested a mixture of 5 probiotic strains in  
18 volunteers exposed to antibiotics and also tested a mixture of 4 probiotic strains in healthy  
19 volunteers with no antibiotic exposure.<sup>50</sup> Zoppi et al. had eight different treatment arms in his  
20 study, and we included four treatment arms in our analysis [*Saccharomyces boulardii* (*S.*  
21 *boulardii*) alone and *Lactobacillus* (*L.*) *rhamnosus* GG alone], a mixture of two probiotics (*L.*  
22 *acidophilus* and *Bifido. bifidum*) and a mixture of three probiotic strains (*L. acidophilus*, *L.*  
23 *rhamnosus* and *Bifido. bifidum*).<sup>51</sup> Orrhage et al. had two treatment arms (*Bifido. longum* alone  
24 and a mixture of *Bifido. longum* and *L. acidophilus*).<sup>52</sup> Larsen et al. tested two single probiotics  
25 (*Bifido. lactis* and *L. acidophilus*) in separate treatment arms.<sup>53</sup> Lidbeck et al. gave either  
26 enoxacin or clindamycin and randomized patients to either *L. acidophilus* or placebo.<sup>54</sup>  
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38 **Normal microbiota assay methods.** Of the 69 treatment arms that did normal microbiota assays,  
39 diverse methods were used to profile the microbiota. Many studies used only standard  
40 microbiological culture assays (37, 54%), while others (28, 40%) used techniques to detect non-  
41 cultivatable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16s rRNA  
42 sequencing) or other PCR techniques. Some studies (4, 6%) used an indirect measure of normal  
43 microbiota, using the Nugent score to diagnose bacterial vaginosis, which relies upon gram stain  
44 of the vaginal secretions, vaginal pH and symptoms to characterize if normal microbiota is  
45 present or absent.<sup>15</sup>  
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3 **Probiotic strains.** In the 69 treatment arms, most (36, 52%) used a single strain of probiotic,  
4 while 14 (20%) tested a mix of two probiotic strains and 19 (28%) tested a mix of three or more  
5 probiotic strains. The distribution of single versus multiple strain probiotics did not significant  
6 vary by the model of study design ( $X^2_2=2.3$ ,  $P=0.32$ ). Of the 15 restorative (Model A) study  
7 arms, 47% used a single strain of probiotic and 53% used multiple strains. Of the 25 treatment  
8 arms with disrupted microbiota at baseline (Model B), 44% used a single strain and 56% used  
9 multiple strains. Of the 29 study arms with undisrupted microbiota (Model C), 62% used a  
10 single strain and 38% used multiple strains.  
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### 20 **Normal microbiota restoration model (Model A)**

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23 The study design with the highest quality capable of documenting the recovery of normal  
24 microbiota due to a probiotic treatment requires that subjects be enrolled with a normal,  
25 undisrupted microbiota, then be exposed to a disruptive factor (e.g. antibiotics, chemotherapy,  
26 disease onset, etc.), followed by probiotic treatment and a follow-up period post-probiotic to  
27 document recovery of the microbiota back to the baseline profiles. We found only 10 studies  
28 (with 15 treatment arms) that fit these criteria (Table 1).<sup>32,34,50-52,54-58</sup> The type of enrolled  
29 subjects varied from healthy volunteers to children with untreated respiratory infections, to  
30 pediatric cancer patients. For subjects with acute infections or cancer, baseline assays were done  
31 prior to the disrupting agent (antibiotics or chemotherapy). The number of subjects given  
32 probiotics averaged 20/study and ranged from 5 to 83. In 93%, the disruptive factor was  
33 antibiotic exposure and in one study, chemotherapy caused the microbiota disruption. Only 8  
34 (53%) of the study arms did an assay during a 1-8 week follow-up period after the probiotic was  
35 discontinued.  
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49 Of the 15 probiotic treatment arms, 7 (47%) showed restoration of the assayed normal  
50 microbiota, 5 (33%) showed partial recovery and 3 (20%) showed no change in the microbiota.  
51 Analysis of the probiotic strain(s) separately found only two probiotic products with more than  
52 one randomized controlled trial. The probiotic mix of *L. acidophilus* and *Bifido. bifidum* showed  
53 a complete restoration in one study, but only a partial recovery in the other. (Strength: strong,  
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Quality: high). The probiotic mix of *L. acidophilus* (2 strains) with *Bifido. bifidum* and *Bifido. animalis* showed complete restoration in one study, but only a partial recovery in the other. (Strength: strong, Quality: high). Five other probiotic products with only one supporting clinical trial showed microbiota restoration (*Bifido. longum*, *Clostridium butyricum*, *L. acidophilus*, mix of *L. acidophilus* with *L. paracasei* and *Bifido. lactis*, and the mix of *L. acidophilus* with *L. paracasei* and *Bifido. bifidum* and two strains of *Bifido. lactis*). (Strength: moderate, Quality: high). Three probiotic products with one supporting clinical trial showed partial restoration (*S. boulardii*, *L. rhamnosus* GG, mix of *L. rhamnosus* with *L. bifidus* and *L. acidophilus*), (Strength: moderate, Quality: high). Only two probiotic products using Model A showed no change in the microbiota (*Bifido. breve* and a mix of *L. acidophilus* and *Bifido. longum*). (Strength: moderate, Quality: high).

Of the 11 probiotic products with claims of 'restores or improves normal microbiota', 10 (91%) were supported by our analysis, but only seven showed complete restoration and five had partial restoration of the microbiota (Table 1). We confirmed that the mixture of *L. acidophilus* and *Bifido. longum* did not show any changes in the microbiota. We disagreed with the conclusions of one of the studies. Wada et al. claimed *Bifido. breve* 'enhanced intestinal anaerobes', but this was only compared to the placebo group.<sup>32</sup> Their data showed chemotherapy is a disruptive event, resulting in more Enterobacteria in the intestine in the placebo group, but there were no significant differences seen by the end of the 8 week follow-up in either the probiotic or the placebo group compared to baseline microbiota levels.

### Disrupted normal microbiota at baseline studies (Model B)

Model B is a study design enrolling subjects with a pre-existing disrupted microbiota related to ongoing disease or conditions. In 25 treatment arms, patients with acute or chronic disease were enrolled and randomized to probiotics or controls and normal microbiota was assayed at enrollment, during treatment and/or post-treatment (Table 2).<sup>33,53,59-80</sup> The number of subjects given probiotics averaged  $23 \pm 16$ /study and ranged from 7-83 participants. The types of pre-existing factors that disrupted the microbiota included atopic dermatitis patients, allergies,

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3 cirrhosis, bacterial vaginosis, irritable bowel syndrome, inflammatory bowel disease (ulcerative  
4 colitis and pouchitis), idiopathic diarrhea, enteral feeding, short-bowel syndrome and colon  
5 cancer. Only 10 (40%) of the study arms did an assay during the post-probiotic follow-up  
6 period.  
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14 Our analysis found 12 (48%) of the study arms supported an alteration or modification of the  
15 microbiome, while 13 (52%) found no significant change in the microbiota. Three of the  
16 probiotics had multiple clinical trials to support the claim of an improvement in the microbiota  
17 due to the probiotic. *S. boulardii* was used in two trials either with enteral fed patients or  
18 patients with active diarrhea and found an improvement in the habitual microbiota in the patients  
19 with active diarrhea<sup>66</sup>, but only showed indirect evidence of short-chain fatty acid changes in the  
20 other study.<sup>65</sup> (Strength: strong, Quality: moderate) A mix of four probiotic strains (2 strains of  
21 *L. rhamnosus*, *P. freudenreichii* + *Bifido. breve*) showed improved microbiota in two clinical  
22 trials.<sup>74,75</sup> (Strength: strong, Quality: moderate) Of four clinical trials testing a mixture of seven  
23 probiotic strains, two showed no significant change in microbiota<sup>77,78</sup>, one showed more  
24 anaerobes post-probiotic treatment<sup>79</sup> and one found a reduction in *Bacteroides* species.<sup>80</sup>  
25 (Strength: strong, Quality: moderate) Three clinical trials determined there were no significant  
26 changes due to *L. plantarum* 299v.<sup>62-64</sup> (Strength: strong, Quality: moderate). Of those probiotics  
27 with only one supporting clinical trial (Strength: moderate, Quality: moderate), two single  
28 probiotic strains (*E. coli* Nissle and *L. casei rhamnosus*) and five different mixtures of probiotic  
29 strains support the claim that the probiotic alters the microbiota (Table 2).  
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46 Of the 25 treatment arms, we confirmed the paper's claim in 14 (56%) of the studies. We agreed  
47 that there was no significant change in the microbiota due to the probiotic in nine treatment arms  
48 only an alteration of the microbiota in five others (Table 2). We disagree with the claimed  
49 outcomes in 11 (46%) of the other treatment arms. In seven treatment arms, it was claimed the  
50 tested probiotic 'restored normal microbiota', but it is uncertain how this conclusion was reached,  
51 since there was no time when a normal undisrupted microbiota was present. Of the seven studies  
52 that claimed their probiotic 'restored' normal microbiota, our analysis determined none were  
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capable of documenting restoration, but we do confirm that probiotics improved or altered the microbiota. Four studies claimed the probiotic 'altered or improved' normal microbiota, but we found no significant differences when post-probiotic and baseline assays were compared for the probiotic groups. Girard-Pipau et al. concluded that *S. boulardii* 'altered normal flora' because more gram positive anaerobes were seen in the probiotic group compared to the controls and an increase in three short-chain fatty acids were observed in the *S. boulardii* group.<sup>65</sup> However, when the analysis is restricted to trends observed in the probiotic group only, no significant differences were observed in pre-probiotic versus post-probiotic microbiota profiles. Venturi et al. concluded that the mix of seven probiotic strains enhanced the concentration of some beneficial strains in the intestines.<sup>77</sup> However, the only strains having a significant increase were those contained in the probiotic mix, and not specifically normal microbiota of the host. As this study did not have an undisturbed microbiota baseline, the increased numbers of Lactobacilli and Bifidobacteria may not have reflected their normal levels. Van der Aa et al. claimed that *Bifido. breve* 'successfully modulates the intestinal flora', but no significant changes were observed in the probiotic group when comparing the baseline to the post-probiotic levels.<sup>59</sup> Odamaki et al. did show an increase in *Faecalibacterium* ssp. and *Bacteroides fragilis* ssp. at the end of *Bifido. longum* BB536 treatment, but the same increase was also observed in the placebo group.<sup>33</sup>

### Undisrupted normal microbiota studies (Model C)

Twenty nine trials enrolled healthy adults who had no disruptive factor present during the study (either no antibiotic or no medication exposure or presence of acute or chronic disease) that might impact normal microbiota, as shown in Table 3.<sup>14,49,50,81-106</sup> The average number of subjects given probiotics was 23/study and ranged from 7 to 160/study. Of the 29 study arms, assays were taken during a follow-up period in only 52%. Not surprisingly, if the normal microbiota was not disturbed, most probiotic treatment arms (25, 86%) did not show a significant change of the microbiota and only 4 (14%) indicated some alteration in the microbiota. Fujiwara et al. cultured seven healthy volunteers and found Enterobacteriaceae and Clostridial species post-*Bifido. longum* was reduced by 10<sup>1</sup>/g compared to baseline (P<0.03), but no other changes in the microbiota were detected.<sup>84</sup> Karlsson et al. found a significant increase in intestinal diversity in nine male volunteers with atherosclerosis given *L. plantarum* 299v, but because

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3 terminal restriction fragment length polymorphism assays were used instead of cultures for  
4 bacterial species, the specific changes in the microbiota species could not be determined.<sup>94</sup> Yang  
5 and Sheu cultured 63 children (55% with *H. pylori*) given a yogurt with *L. acidophilus* and  
6 *Bifido. lactis* but only found a decrease in *E. coli* counts in the *H. pylori* negative children sub-  
7 group, no significant changes in normal microbiota was found in the *H. pylori* positive  
8 children.<sup>100</sup> Kubota et al. assayed 29 subjects with Japanese cedar pollen allergy and found milk  
9 fermented with *L. rhamnosus* GG and *L. gasseri* TMC0356 suppressed microbiota changes (less  
10 intestinal profile changes), but could not determine specific bacterial species changes due to the  
11 type of assay used (FISH and TRFLP).<sup>103</sup>  
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23 Of the seven studies that claimed their probiotic(s) 'restored or altered' the normal microbiota, we  
24 confirmed only four claims, but disagree with three studies. Sierra et al. claimed *L. salivarius*  
25 given to 20 healthy adults 'improved gut microbiota', but only increased levels of Lactobacilli  
26 were found and no other changes in normal microbiota species were detected. The only other  
27 evidence was indirect from changes observed in immune parameters.<sup>96</sup> He et al. claimed a  
28 mixture of *Bifido. longum* and *Bifido. animalis* 'modified' microbiota, but changes were seen  
29 only during the yogurt administration and not after the one week follow-up period.<sup>99</sup> Vitali et al.  
30 claimed that the mixture of four Lactobacilli strains and three Bifidobacteria strains 'modulated  
31 vaginal microbiota', but the only significant changes were due to an increase in the bacterial  
32 species contained in the probiotic mixture.<sup>14</sup>  
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44 Of the probiotics supported by multiple clinical trials (*Bifido. animalis*, *Bifido. longum*, *L. casei*,  
45 *L. plantarum* 299v, the mixture of *Bifido. animalis* and *Bifido. lactis*), 13 (87%) support there is  
46 no significant change in normal microbiota if the microbiota is not disrupted. [Strength: strong,  
47 Quality: low)  
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#### 55 **Association of clinical efficacy and normal microbiota restoration**

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Few studies concurrently compared clinical efficacy and the ability to restore or improve normal microbiota after dysbiosis. A synthesis of the literature of RCT for eight common disease indications was performed and the overall net strength was ranked. Probiotics with the ability to restore normal microbiota were frequently supported by RCTs for efficacy, as shown in Table 4. Of the 10 probiotics with evidence for restoration, 7 (70%) also had at least one RCT testing for at least one of the eight diseases, while 30% did not have any supportive RCTs for efficacy. Of the 7 probiotics with associated RCTs, only two probiotics (*S. boulardii* and *L. acidophilus*) have strong evidence for efficacy across most of the disease indications, while five probiotics with the ability to restore the microbiota had weak or no evidence of efficacy. For example, *S. boulardii*, which has studies supporting restoration, has strong evidence for clinical efficacy for AAD (ranked ++: 11 RCTs had significant results and 6 had non-significant results), CDI (ranked ++: had two RCTs with significant results), IBD (ranked ++: had two RCTs with significant results), IBS (ranked 0: had one RCT with significant efficacy and one RCT with non-significant results), TD (ranked +: 3 RCTs with significant efficacy and 2 with non-significant efficacy), *H. pylori* eradication (ranked -: 2 RCTs with significant results and 4 with non-significant results) and no studies for BV. *L. acidophilus*, which partially restored the microbiota in a study, is associated with clinical efficacy for AAD, IBS and BV, but not for TD or eradication of *H. pylori* and treatment of acute pediatric diarrhea (ranked ++: had 19 RCTs with significant protection and five with non-significant results). In contrast, *L. rhamnosus GG*, another probiotic capable of restoring microbiota, is often cited in meta-analysis as having significant efficacy for AAD. Our results of an updated review of the literature indicate a net weak evidence rating for clinical efficacy across all disease indications: AAD (ranked -: 3 RCTs had significant results and 6 had non-significant results), CDI (ranked -: two RCTs with non-significant results), IBD (ranked -: one RCT with non-significant results), IBS (ranked 0: 2 RCTs with significant efficacy and two RCTs with non-significant results), TD (ranked 0: one RCT with significant efficacy and one with non-significant efficacy), *H. pylori* eradication (ranked -: 3 RCTs with non-significant results), no RCTs for BV and treatment of acute pediatric diarrhea (ranked ++: 10 RCTs with significant efficacy and one with non-significant findings).

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3 Efficacy trials were not done as frequently for probiotics shown to only have the ability to alter  
4 or improve, but not restore, the microbiota after dysbiosis. Of nine probiotics that can alter the  
5 microbiota, 6 (67%) have supporting RCTs for at least one disease, but the diversity of  
6 investigated diseases was more limited. *L. casei* had moderate net strength for AAD and  
7 bacterial vaginosis, but was neutral for the ability to eradicate *H. pylori* and other disease  
8 indications were not tested in RCTs with *L. casei*. The probiotic mixture of *L. reuteri* and *L.*  
9 *fermentum* has strong evidence for bacterial vaginosis, but not for any other disease indications  
10 listed in Table 4.  
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21 Of the eight probiotics not capable of altering or restoring normal microbiota, only *L. plantarum*  
22 299v had RCTs for AAD and IBS, both with net negative or weak strength of clinical efficacy.  
23 *Bifido. lactis* and the mixture of *L. rhamnosus* and *L. reuteri* had net neutral rankings for efficacy  
24 for the treatment of acute pediatric diarrhea. The other four probiotic products with no effect on  
25 normal microbiota lacked any RCTs for clinical efficacy. Studies with *B. clausii* did not assay  
26 for normal microbiota and had non-significant trial results for *H. pylori* eradication and the  
27 treatment of pediatric diarrhea.  
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35 Of the six probiotics with only pharmacokinetic data on the probiotic itself and no other  
36 investigation of other normal microbiota strains, five had RCTs showing varying net efficacies  
37 for different disease indications, as shown in Table 4.  
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42 Six popular probiotics (*Bacillus clausii*, *Bifido. infantis*, *L. reuteri*, *L. acidophilus* + *L. helveticus*,  
43 *L. acidophilus* + *L. casei* and *L. acidophilus* + *Bifido. animalis*) have only clinical efficacy  
44 RCTs, but have not published studies investigating their role in restoring or improving the  
45 normal microbiota.  
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## 52 Discussion

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54 Developing and evaluating health claims for probiotics is an important issue and is now  
55 identified as a priority for research by several international organizations, including the World  
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3 Gastroenterology Organization<sup>107</sup> and the American Society for Nutrition.<sup>2</sup> The U.S. Food and  
4 Drug Administration has struggled with appropriate evidence-based health claims for probiotic  
5 products and recommends the use of structure/function claims, such as "maintains bowel  
6 regularity", but the claim for restoring normal microbiota is under debate.<sup>108</sup> The European Food  
7 Safety Authority (EFSA) provides guidance materials that recommend health claims for  
8 probiotics should have beneficial physiological effects and have appropriate scientific trials to  
9 substantiate the health claims.<sup>3</sup> Acceptable claims for intestinal health include functional claims  
10 (improved transit time, softer stool consistency, reduction in gastrointestinal discomfort, defense  
11 against pathogens) and changes related to gastrointestinal microbiota. As it is currently not  
12 possible to define a standard normal microbiota profile, the EFSA recommends functional claims  
13 for the restoration of normal microbiota should be accompanied by a beneficial physiological or  
14 clinical outcome.<sup>3</sup> In addition, because the efficacy and mechanisms are strain-specific and may  
15 vary by probiotic strain, the evidence must be analyzed for each probiotic product  
16 individually.<sup>5,6,9,109,110</sup>  
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30 When the literature on probiotics and its impact on the normal microbiota was reviewed from 69  
31 different treatment arms, the evidence shows probiotics restored normal microbiota in only 10%  
32 of the trials, probiotics altered normal microbiota in 30% and no change in the normal microbiota  
33 was found (60%).  
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39 An underappreciated finding was the influence that study design and study populations have on  
40 the interpretation of study results. In this review, five different types of study designs were  
41 found in the literature relating to probiotics and dysbiosis. The most common study type was a  
42 randomized controlled trial testing the efficacy and safety outcomes in patients, but these trials  
43 did not typically document the impact of the probiotic on the normal microbiota of the patient.  
44 The second type of study design was for pharmacokinetic studies (documenting recovery of oral  
45 dose of probiotic or increase in probiotic strains post-treatment compared to pre-treatment or  
46 clearance of the probiotic). Even though these kinetic studies did not assay for non-probiotic  
47 strains, some extrapolated their results and concluded some effect or improvement of the normal  
48 microbiota was observed by their probiotic.<sup>19,111</sup> These two first types of study designs do not  
49 support evidence-based conclusions for the restoration or alteration of the normal microbiota and  
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3 were excluded from this review. The third type of study design started with inappropriate  
4 baselines (baseline samples taken after normal microbiota had been disrupted by chronic  
5 disease). For patients with established chronic diseases, there is no “normal microbiota” baseline  
6 in either the probiotic or the control group. Even if baselines are taken during remission, the  
7 microbiota may still be impacted by chronic disease or acute diarrhea. Studies of probiotics in  
8 chronic diseases or acute disease typically report on ‘pre-probiotic treatment’ and ‘post-probiotic  
9 treatment’ and may show significant shifts in microbial species, but it is uncertain if this reflects  
10 a true re-establishment of normal microbiota profiles. The fourth type of study design enrolled  
11 healthy volunteers, who were not challenged with antibiotics (so no normal microbiota  
12 disruption occurred), and show only the effect of probiotics on a healthy microbiota (typically  
13 mild or no effects). The fifth type of study design had normal microbiota assayed at least twice  
14 (at baseline, which was before exposure to a disruptive event or probiotics and then again during  
15 or post-probiotic treatment) to show actual recovery of assayed normal microbiota back to  
16 baseline levels. Control groups were not required for our assessment of the impact of probiotics  
17 on microbiota, but control groups can document the degree normal microbiota is disrupted by  
18 inciting agents (antibiotic, disease onset, etc.).  
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35 Five single strain probiotics (*Bifido. longum*, *Clost. butyricum*, *L. acidophilus*, *L. rhamnosus* and  
36 *S. boulardii*) and four probiotic mixtures (*L. acidophilus* + *Bifido. bifidum*, *L. rhamnosus* + *L.*  
37 *bifidus* + *L. acidophilus*, *L. acidophilus* + *L. paracasei* + *Bifido. lactis*, *L. acidophilus*, 2 strains,  
38 *Bifido. bifidum*, *Bifido. animalis*) documented either complete or partial recovery of normal  
39 microbiota (Model A). Only two probiotic mixtures [(2 strain mixture: *L. acidophilus* + *Bifido.*  
40 *bifidum*) and (4 strain mixture: *L. acidophilus*, 2 strains, *Bifido. bifidum*, *Bifido. animalis*)] were  
41 supported by a confirmatory study. Evidence that probiotics may alter or improve normal  
42 microbiota (Model B) was found for three single strain probiotics (*E. coli* Nissle, *S. boulardii*  
43 and *L. casei rhamnosus*) and seven mixtures of 2-7 probiotic strains. Of these ten probiotics  
44 finding alteration of the microbiota, only three had multiple trials [*S. boulardii*, and a four strain  
45 mixture (2 strains of *L. rhamnosus* + *P. freudenreichii* + *Bifido. breve*), and a seven strain  
46 mixture (4 Lactobacilli and 3 Bifidobacteria strains)], but only one had consistent results  
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3 showing improvements in the microbiota.<sup>74,75</sup> Clearly, more than one study is needed to confirm  
4 the impact of a probiotic on the normal microbiota.  
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10 Of the 19 probiotic strains (or mixtures) studied in healthy volunteers who were not exposed to  
11 disruptive factors, no change in the normal microbiota was observed for 79%, indicating the  
12 robustness of the microbiota.  
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18 Improvement in the normal microbiota by specific probiotic strains seemed to be associated with  
19 better clinical endpoints. Within eight common diseases typically treated with probiotics, more  
20 trials with significant efficacy were associated with probiotic strains shown to restore the normal  
21 microbiota, and only one trial with significant efficacy was found for probiotics that did not alter  
22 the microbiota. However, few probiotics had efficacy trials for all eight diseases and many did  
23 not have any efficacy trials.  
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33 Some probiotics which have published efficacy trials for various diseases did not have studies  
34 investigating the effect of the probiotic on normal microbiota: *Bacillus clausii*, *Bifido. infantis*, *L.*  
35 *brevis*, *L. reuteri*, mix of 2 strains (*L. acidophilus* + *L. helveticus*), mix of 2 strains (*L.*  
36 *acidophilus* + *L. casei*) or (*L. acidophilus* + *Bifido. animalis*), mix of 4 strains [*L. rhamnosus* (2  
37 strains), *Propionibacterium freudenreichii* + *Bifido. animalis*] and mix of 7 strains (*L.*  
38 *sporogens*, *L. bifidum*, *L. bulgaricus*, *L. thermophilus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*).  
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### 48 **Comparison of results with other studies**

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50 Other reviews in the literature of health claims for probiotics relating to changes in the normal  
51 microbiota have focused on the broad issues of regulatory standardization of health claims, the  
52 use of proper study designs and the challenge of defining biomarkers for a 'healthy  
53 microbiota'.<sup>3,29,112</sup> Donovan et al. recommends that health claims for probiotics be supported by  
54 well-conducted human trials in the targeted population.<sup>2</sup> These reviews also recommend that gut  
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3 biomarkers need to be correlated with clinical endpoints, however none of these reviews  
4 attempted to do so.<sup>29,112</sup> No prior review has attempted to analyze the association between  
5 probiotic strains and their impact on normal microbiota by stratifying on the quality of study  
6 design.<sup>111</sup> This review addressed these concerns by analyzing probiotic strains by the quality of  
7 the study design and only including trials that assessed the normal microbiota (either by  
8 microbial culturing or molecular strain biomarkers) and assessed the degree of dysbiosis  
9 improvement with clinical outcomes for each probiotic strain.  
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### 20 **Opportunities for future research**

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22 Most of the studies (80%) using Model A to document restoration of the normal microbiota only  
23 used microbiologic culturing techniques, which can only detect those organisms that grow in  
24 culture. Use of the more advanced molecular metagenomic techniques have found that culturing  
25 alone misses up to 95% of these organisms.<sup>21,22</sup> The use of the metagenomic techniques was  
26 more common in the studies using Model B (48%) and Model C (45%) study designs, which  
27 only addresses potential alteration of the microbiota. Characterization of the microbiota is a  
28 complex issue and a comprehensive accounting of all the bacterial and fungal strains in the body  
29 is beyond our current capabilities. Therefore, any studies of changes to the microbiota are  
30 incomplete at best, but general trends in bacterial phylotypes can be documented using DNA  
31 probes and metagenomic techniques. Differential detection bias may be present due to the  
32 variety of assays used in these studies and should be accounted for in future studies.  
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45 Another suggestion for future studies is to include an appropriate follow-up time period post-  
46 probiotic administration. Fewer than half of the reviewed trials did assays for normal microbiota  
47 during an appropriate follow-up period. As it has been shown that recovery from a disrupting  
48 factor can be prolonged (typically eight weeks),<sup>7,8</sup> and studies that failed to find microbiota  
49 recovery might have detected a return to normal baseline levels if a sufficiently long time was  
50 given for the recovery to have occurred. Future studies should strive to allow time for the  
51 restoration of the normal microbiota to occur.  
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## Strengths and weaknesses

The strengths of this review included the completeness of the search strategy, which reviewed multiple citation databases, trial registries and author searches, use of established PRISMA protocols for reviews and the use of an outcome classification scheme for different degrees of assessment for microbial recovery. This analysis controlled the confounding effects of different study populations and study designs present in the literature. Pharmacokinetic studies of just the probiotic strain(s) itself were excluded and only trials that assayed other species found in the microbiota were included. By applying a standard definition for 'restoring' versus 'improving' normal microbiota, it is possible to distinguish significant differences by the type of study designs used and differential effects of the different probiotic strains. Limitations of this review include pooling trials from different populations (adult versus pediatric) and different probiotic doses and regimens used. Incomplete retrieval of all studies assessing the effect that probiotics have on human microbiota is also a potential limitation of any literature search. Another limitation is that dysbiosis improvement and clinical efficacy for probiotic strains is also indirectly associated, no direct cause and effect relationship was possible with the types of studies done.

## Conclusion

The challenges in recommending a specific probiotic to patients who need to restore or improve their normal microbiota after a disrupting event occurs is two-fold: one is the diversity of probiotic products available and second is the varying strength of evidence provided by clinical trials using different outcome measures and study designs. By grouping studies into three groups that result in three different degrees of probiotic effect (restoration, improvement or no change), an overview of the body of evidence is possible. By comparing the strength of the clinical evidence for common diseases by the degree to which the probiotics could impact the restoration of the normal microbiota, it became obvious that those probiotics with a greater ability to restore the microbiota are associated with the strongest strength of clinical efficacy. While this evidence only indirectly links clinical efficacy with the ability to restore the microbiota, the overall review of the evidence shows this is an important mechanism of action for probiotics. What becomes

obvious is that more studies are required to conclude which probiotic strains have a beneficial impact on the normal microbiota, as most strains have only a single clinical trial and many probiotic products overstate the strength of their claim to restore normal microbiota.

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17 **Figure 1.** Time sequence of events and three models of study designs determining three different  
18 degrees of dysbiosis correction by probiotics.  
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**Figure 2.** Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics.

Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota

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**Table 1. Model A:** Evidence-based data for restoration of normal microbiota (NM) for 12 probiotics from 10 studies (15 treatment arms).

Probiotic	Reference	No. treated with probiotic	Type(s) of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment (wks)	Claims stated in papers	Evidence-based claim
<i>Bifido. breve</i>	Wada 2010 <sup>32</sup>	19	FISH	pediatric cancer patients	chemotherapy	8	enhances anaerobes	no change
<i>Bifido. longum</i>	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	restores	restores
<i>Clost. butyricum</i>	Seki 2003 <sup>34</sup>	83	culture	pediatric respiratory or GI infections	antibiotics	0	restores	restores
<i>L. acidophilus</i>	Lidbeck 1988 <sup>54</sup>	5	culture	healthy volunteers	enoxacin or	1	restores only in enoxacin	restores only in enoxacin,
		5	culture	volunteers	clindamycin	1	no change	no change in clindamycin
<i>L. rhamnosus GG</i>	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>S. boulardii</i>	Zoppi 2001 <sup>51</sup>	6	culture	pediatric respiratory infections	ceftriaxone	0	improves	partially restores
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	Black 1991, <sup>55</sup> Zoppi 2001 <sup>51</sup>	10,	culture,	healthy volunteers,	ampicillin,	2,	recovers more rapidly,	restores,
		7	culture	pediatric respiratory	ceftriaxone	0	less change	partially restores
<i>L. acidophilus</i> + <i>Bifido. longum</i>	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	no change	no change
<i>L. rhamnosus</i> + <i>L. bifidus</i> + <i>L. acidophilus</i>	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido lactis</i>	Jernberg 2005 <sup>56</sup>	4	culture PCR TRFLP	healthy volunteers	clindamycin	2	restores	restores
<i>L. acidophilus</i> + <i>L. acidophilus</i> + <i>Bifido. bifidum</i> + <i>Bifido animalis</i>	Madden 2005, <sup>57</sup> Plummer 2005 <sup>58</sup>	15,	culture,	<i>H. pylori</i> +,	amoxicillin + metronidazole,	2,	restores,	restores,
		76	culture	<i>H. pylori</i> +	amoxicillin + clarithromycin	2	restores more rapidly	partially restores
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. bifidum</i> + <i>Bifido. lactis</i> + <i>Bifido. lactis</i>	Engelbrek-tson 2006 <sup>50</sup>	20	culture PCR TRFLP	healthy volunteers	augmentin	2	restores	restores

**Table 2. Model B:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 18 probiotics from 24 studies (25 treatment arms) with disturbed microbiota at baseline.

Probiotic	Reference	No. treated with probiotic	Type(s) of assay for NM	Pre-existing disrupting factor	Follow-up time	Claims stated in papers	Evidence-based claim	Type of change found in NM
<i>Bifido. breve</i>	Van der Aa 2010 <sup>59</sup>	46	FISH	Atopic dermatitis	0	modulates NF	no change	--
<i>Bifido. lactis</i>	Larsen 2011 <sup>53</sup>	17	PCR	Atopic dermatitis	0	no change	no change	--
<i>Bifido. longum</i>	Odamaki 2007 <sup>33</sup>	22	TRFLP PCR	Cedar pollen allergy	4 wk	maintains NF	no change	--
<i>E. coli</i>	Lata 2007 <sup>60</sup>	22	culture	liver cirrhosis	0	restores	improves	more Bifido. & Lacto.
<i>L. acidophilus</i>	Larsen 2011 <sup>53</sup>	17	PCR	atopic dermatitis	0	no change	no change	--
<i>L. casei rhamnosus</i>	Petricevic 2008 <sup>61</sup>	83	Nugent scores	bacterial vaginosis	4 wk	restores	improves	improved Nugent scores
<i>L. plantarum 299v</i>	Nobaek 2000, <sup>62</sup> Klarin 2005, <sup>63</sup> Klarin 2008 <sup>64</sup>	25, 17, 22	culture, culture, culture	IBS, enterally-fed, antibiotics	4 wk, 0, 0	no change, no change, no change	no change, no change, no change	--
<i>S. boulardii</i>	Girard 2002, <sup>65</sup> Swidsinski 2008 <sup>66</sup>	10, 20	culture, FISH	enterally-fed, active diarrhea	9 d, 3 wk	alters NF, improves	no change, improves	-- more 'habitual microbiota'
<i>L. reuteri</i> + <i>L. fermentum</i>	Reid 2001 <sup>67</sup>	33	Nugent scores	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores
<i>L. rhamnosus</i> + <i>L. fermentum</i>	Reid 2003 <sup>68</sup>	31	Nugent scores and culture	bacterial vaginosis	30 d	restores	improves	improved Nugent scores
<i>L. plantarum</i> + <i>Bifido bifidum</i>	Kirpich 2008 <sup>69</sup>	32	culture	colon cancer	0	restores	improves	more <i>E. coli</i> and <i>Enterococci</i>
<i>L. rhamnosus</i> + <i>L. reuteri</i>	Hummelen 2010 <sup>70</sup>	23	Nugent score	bacterial vaginosis	0	no change	no change	--
<i>L. casei</i> + <i>Bifido breve</i>	Uchida 2007 <sup>71</sup>	4	culture	short bowel syndrome	0	no change	no change	--
<i>L. brevis</i> + <i>L. salivaris</i> + <i>L. plantarum</i>	Mastromarini 2009 <sup>72</sup>	19	Nugent score	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores
<i>L. paracasei</i> + <i>L. acidophilus</i> + <i>Bifido. animalis</i>	Roessler 2012 <sup>73</sup>	30	PCR	atopic dermatitis	0	no change	no change	--

<i>L. rhamnosus</i> + <i>L. rhamnosus</i> + <i>P. freudenreichii</i> + <i>Bifido. breve</i>	Kajander 2005, <sup>74</sup>	41,	PCR,	IBS,	0,	restores,	improves,	Improved similarity index
	Lyra 2010 <sup>75</sup>	22	PCR	IBS	0	alters	alters	More Clostridia and Rumino- coccus
<i>L. acidophilus</i> + <i>L. plantarum</i> + <i>L. rhamnosus</i> + <i>Bifido. bifidum</i>	Wong 2013 <sup>76</sup>	7	PCR	liver disease	0	improves	alters	Less Firmicutes, more Bacterio- detes
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp.</i> <i>bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Venturi 1999, <sup>77</sup>	20,	culture	ulcerative colitis,	15 d,	enhances,	no change,	--
	Brigidi 2001, <sup>78</sup>	10,	culture & PCR	IBS,	10 d,	no change,	no change,	--
	Kuhbacher 2006 <sup>79</sup>	10	FISH	pouchitis	0	altered richness	altered	More anaerobes
	Ng 2013 <sup>80</sup>	10	PCR	IBS	0	modulates	altered	Less Bacteroides

\*disruption of normal microbiota at baseline shown by significant differences compared to control (non-diseased) population.

**Table 3. Model C:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 19 probiotics in healthy volunteers enrolled in 29 studies (29 treatment arms) in studies with no disruptive exposures.

Probiotic	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment	Claims stated in papers	Evidence-based claim
<i>Bifido. animalis (lactis)</i>	Rochet 2008, <sup>49</sup> Oswari 2013 <sup>81</sup>	12, 160	FISH PCR	healthy volunteers	none, none	10 d, 6 mon	no change, no change	no change, no change
<i>Bifido. bifidum</i>	Langhendries 1995 <sup>82</sup>	20	culture	healthy volunteers	none	0	no change	no change
<i>Bifido. longum</i>	Benno 1992, <sup>83</sup> Fujiwara 2001, <sup>84</sup> Harmsen 2002 <sup>85</sup>	5, 7, 14	culture, culture, FISH	healthy volunteers	none, none, none	0, 30 d, 0	no change, alters, no change	no change, alters, no change
<i>L. casei</i>	Guerin 1998, <sup>86</sup> Rochet 2006, <sup>87</sup> Rochet 2008 <sup>88</sup>	12, 12, 7	culture, FISH, FISH	healthy volunteers	none, none, none	1 wk, 10 d, 0	no change, no change, no change	no change, no change, no change
<i>L. johnsonii</i>	Brunser 2006 <sup>89</sup>	32	culture & FISH	healthy volunteers	none	2 wk	no claim	no change
<i>L. plantarum 299v</i>	Goossens 2003, <sup>90</sup> Goossens 2005, <sup>91</sup> Goossens 2006, <sup>92</sup> Berggren 2003, <sup>93</sup> Karlsson 2010 <sup>94</sup>	11, 32, 15, 33, 9	culture, culture, culture, culture, TRFLP	healthy, healthy, colonic polyps, healthy, atherosclerosis	none, none, none, none, none	3 wk, 4 wk, 0, 0, 0	no change, no change, no change, no change, alters	no change, no change, no change, no change, alters
<i>L. rhamnosus GG</i>	Gueimonde 2006 <sup>95</sup>	29	PCR	healthy volunteers	none	0	no change	no change
<i>L. salivarius</i>	Sierra 2010 <sup>96</sup>	20	culture	healthy volunteers	none	0	improves	no change
<i>S. boulardii</i>	Vanhoutte 2006 <sup>97</sup>	30	PCR	healthy volunteers	none	0	no change	no change
<i>Bifido. animalis + Bifido. longum</i>	Zhong 2006, <sup>98</sup> He 2008 <sup>99</sup>	11, 11	FISH, FISH	healthy volunteers	none	7 d, 7d	no change, modifies	no change, no change
<i>L. acidophilic + Bifido. lactis</i>	Yang 2012 <sup>100</sup>	63	culture	healthy but 55% <i>H. pylori</i> +	none	0	restores	alters
<i>L. rhamnosus GG + Bifido. longum</i>	Mah 2007 <sup>101</sup>	20	FISH	healthy neonates	none	6 mon	no change	no change
<i>L. rhamnosus GG + Bifido. lactis</i>	Rafter 2007 <sup>102</sup>	38	culture	colon cancer patients or at risk	none	0	no change	no change

<i>L. rhamnosus GG</i> + <i>L. gasseri</i>	Kubota 2009 <sup>103</sup>	14	culture FISH TRFLP	healthy, allergy patients	none	0	suppressed changes	alters
<i>L. paracasei</i> + <i>L. paracasei</i> + <i>L. gasseri</i>	Morelli 2003 <sup>104</sup>	12	culture	healthy volunteers	none	3 d	no claims	no change
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i>	Sullivan 2009 <sup>105</sup>	15	culture	chronic fatigue patients	none	4 wk	no change	no change
<i>L. rhamnosus</i> + <i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. animalis</i>	Engelbrektsen 2006 <sup>50</sup>	22	culture TRFLP PCR	healthy volunteers	none	2 wk	no change	no change
<i>Bifido. animalis</i> + <i>L. delbrueckii</i> + <i>L. delbrueckii</i> + <i>L. lactis</i>	McNulty 2011 <sup>106</sup>	7	PCR	healthy twins volunteers	none	4 wk	no change	no change
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp.</i> <i>bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Vitali 2012 <sup>14</sup>	15	PCR	healthy pregnant volunteers	none	0	modulates	no change

Abbreviations: FISH, fluorescence *in situ* hybridization analysis; TRFLP, terminal restriction fragment length polymorphism analysis; PCR, polymerase chain reaction

**Table 4.** Comparison of the ability of probiotic to restore or improve dysbiosis with ranked clinical efficacy for various disease indications.

Probiotic	Restored normal microbiota*	Altered normal microbiota*	Ranked net evidence for efficacy*							
			AAD	CDI	IBD	IBS	TD	H pylori	Vaginitis/BV	Acute Ped diar
<b>Restores microbiota</b>										
<i>Clostr. butyricum</i>	yes	nd	-						-	
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	yes	nd	<b>0</b>	-						
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i>	yes	nd				-				
<i>Bifido. longum</i>	yes	no			-	+				
<i>L. acidophilus</i> + <i>L. acidophilus</i> + <i>Bifido. bifidum</i> + <i>Bifido. animalis</i>	yes	nd								
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i> (2)	yes	no								
<i>S. boulardii</i>	partial	yes	++	++	++	<b>0</b>	+	-		++
<i>L. rhamnosus GG</i>	partial	nd	-	-	-	<b>0</b>	<b>0</b>	-	<b>0</b>	++
<i>L. acidophilus</i>	partial	no	++			++	-	-	+	<b>0</b>
<i>L. acidophilus</i> + <i>L. bifidus</i> + <i>L. rhamnosus</i>	partial	nd								
<b>Alters microbiota</b>										
<i>E. coli</i>	nd	yes			-					+
<i>L. casei</i> (DN114001 or Lcr35)	nd	yes	+					<b>0</b>	+	++
<i>L. reuteri</i> + <i>L. fermentum</i>	nd	yes							++	
<i>L. rhamnosus</i> + <i>L. fermentum</i>	nd	yes								



<i>L. plantarum</i> + <i>Bifido. bifidum</i>	nd	yes								
<i>L. rhamnosus</i> + <i>L. rhamnosus</i> + <i>P. freudenreichii</i> + <i>Bifido. breve</i>	nd	yes				++				
<i>L. acidophilus</i> + <i>L. plantarum</i> + <i>L. rhamnosus</i> + <i>Bifido bifidum</i>	nd-	yes								
<i>L. brevis</i> + <i>L.</i> <i>salivarius</i> + <i>L.</i> <i>plantarum</i>	nd	yes							+	
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp.</i> <i>bulgaricus</i> + <i>L. plantarum</i> , <i>Bifido. longum</i> , <i>Bifido. infantis</i> , <i>Bifido. breve</i>	nd	yes	-		++	+				++
<b>No effect on microbiota</b>										
<i>B. clausii</i>	nd	nd							-	-
<i>L. plantarum 299v</i>	nd	no	-	-		-				
<i>Bifido. lactis</i>	nd	no	+							0
<i>Bifido. breve</i>	no	no								
<i>L. acidophilus</i> + <i>Bifido. longum</i>	no	--								
<i>L. rhamnosus</i> + <i>L. reuteri</i>	nd	no								0
<i>L. casei</i> + <i>Bifido. breve</i>	nd	no								
<i>L. paracasei</i> + <i>L. acidophilus</i> + <i>Bifido animalis</i>	nd	no								
<b>Pharmacokinetic only</b>										
<i>L. reuteri</i>	nd	nd								+
<i>L. johnsonii Lal</i>	nd	nd				-			+	
<i>L. salivarius</i>	nd	nd					-			
<i>Bifido. animalis</i>	nd	nd					0			
<i>Bifido. bifidum</i>	nd	nd					+			
<i>L. rhamnosus</i> + <i>Bifido. longum</i>	nd	nd								

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3 \* **Rank:** ++,  $\geq 2$  net RCTs (randomized controlled trials) with significant protective efficacy; +,  
4 only one net protective RCT; 0, equal number of significant and non-significant RCTs; -,  $\geq 1$  net  
5 non-significant RCT. Blank indicates no RCT done for the disease indication.  
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8  
9 **Abbreviations:** nd, not determined; AAD, antibiotic associated diarrhea; CDI, Clostridium  
10 difficile infections; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; TD,  
11 traveler's diarrhea; BV, bacterial vaginosis; Acute Ped Diar, treatment of acute pediatric  
12 diarrhea.  
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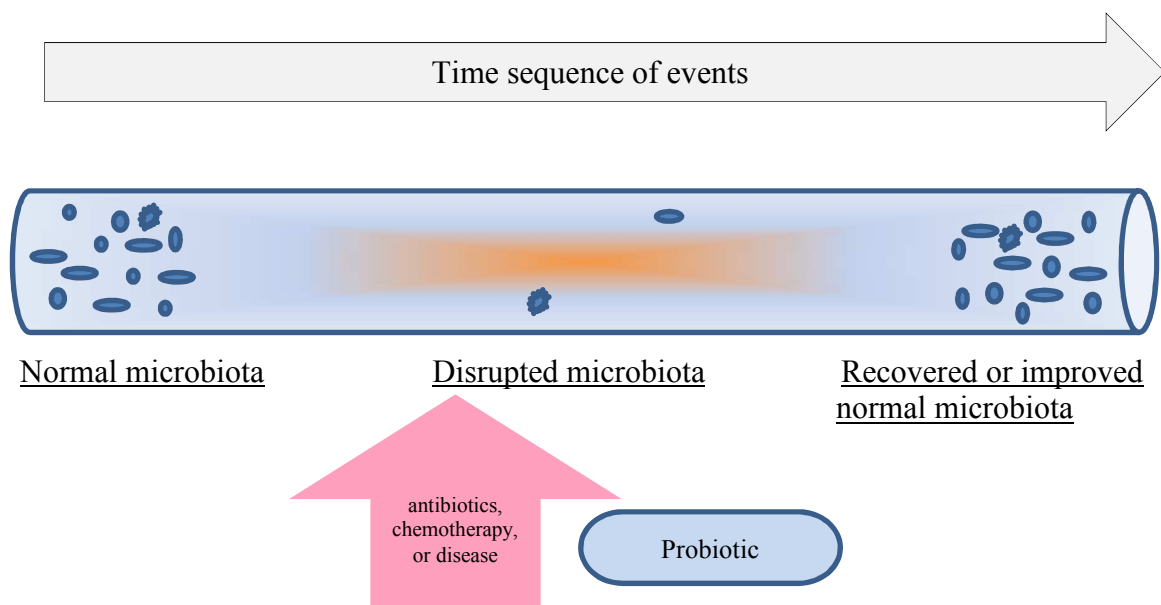
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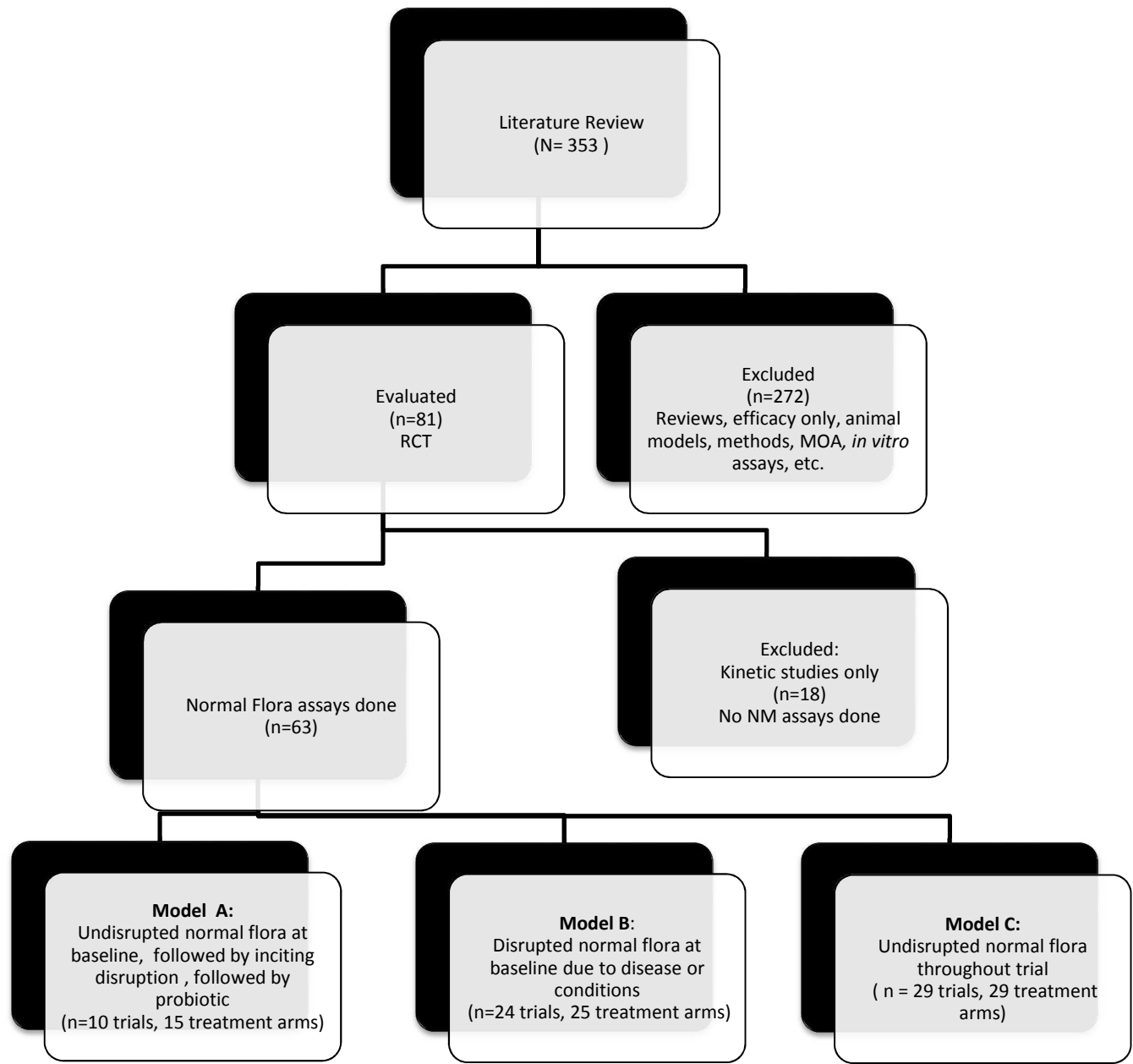
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Model	Type of population enrolled	Dysbiosis	Time microbiota disrupted	Probiotic or control intervention	Potential outcomes
A	Healthy volunteers or at-risk patients	no	post-baseline	preventive	restoration
B	Patients with active disease at enrollment	yes	pre-baseline	treatment	altered or improved
C	Healthy volunteers	no	not disrupted	preventive	no change

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## PRISMA Checklist

Item	Topic	Reported on page
1	Title includes systematic review	1
2	Structured abstract/summary	2
3	Introduction: rationale for review	4
4	Introduction: questions addressed (PICOS)-participants, interventions, comparisons, outcomes, study design	4-5
5	Methods: Registration for protocol	2
6	Methods : Eligibility criteria	6
7	Methods: Information sources & databases	5-6
8	Methods: search strategy	5
9	Methods: study selections (screening, eligibility)	6
10	Methods: method of data extraction	6
11	Methods: define all data items & variables sought	6-8
12	Methods: Methods assessing for risk of bias	8
13	Methods: Principal summary outcome measures	6-7
14	Methods: Synthesis of results, pooling methods	6-7
15	Methods: any assessment of risk for bias (publication bias)	8
16	Methods: Any subgroup or sensitivity analysis	na
17	Results: Number studies screened flow diagram	Figure 2
18	Results: Study characteristics (study size, follow-up)	Tables 1-3
19	Results: Data on risk for bias (see #12)	11-15
20	Results: Outcomes for each study	Tables 1-3
21	Results: synthesis of results, data on each meta-analysis, pooled data	not pooled
22	Results: results of risk of bias across studies (see #15)	na
23	Results: present additional analysis data (see #16)	Table 4
24	Discussion: Summary of main findings	18-20
25	Discussion: Limitations	22
26	Discussion: Conclusions	22
27	Funding: describe funding sources	23

# BMJ Open

## A systematic review for evidence-based studies of probiotics supporting a claim for microbial dysbiosis.

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## A systematic review for evidence-based studies of probiotics supporting a claim for microbial dysbiosis.

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**Disclaimer:** *The findings and conclusions in this study are those of the author and do not represent the official position of the University.*

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**Word Count** 6719

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**Tables:** 4

## ABSTRACT

**Objective:** To assess the evidence for the claim probiotics can correct dysbiosis of the normal microbiota resulting from disease or disruptive events.

**Setting:** Systematic review of published clinical trials of patients receiving a probiotic intervention for the prevention or treatment of various diseases.

**Data sources:** Sources searched (1985-2013): PubMed, EMBASE, Cochrane Database of Systematic Reviews, CINAHL, AMED, and ISI Web of Science. Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.

**Review methods:** Included studies were randomized clinical trials of probiotic interventions having microbiologic assays. Studies were evaluated following PRISMA guidelines for specific probiotic strains. A standard data extraction form was used to collect the raw data.

**Outcome measures:** The primary outcome is the degree of microbiota correction by specific probiotic strains. Secondary outcome was the association between the degree of dysbiosis correction and clinical efficacy.

**Results:** The review of the literature found three distinct study designs: Model A (restoration) assayed patients enrolled with a healthy, undisturbed microbiota and then assayed post-disruptive event and probiotic therapy; Model B (alteration) assayed patients with pre-existing disrupted microbiota and then post-probiotic therapy; Model C (no dysbiosis) assayed volunteers with no disruptive event pre and post-probiotic. From a total of 63 trials, 83% of the probiotic products using Model A restored the microbiota, 56% using Model B improved the microbiota and only 21% using Model C had any effect on microbiota. Clinical efficacy was more commonly associated with strains capable of restoration of the normal microbiota.

**Conclusions:** The ability to assess the degree of dysbiosis improvement is dependent upon the enrolled population and the timing of microbiologic assays. The functional claim for correcting dysbiosis is poorly supported for most probiotic strains and requires further research.

**Systematic review registration:** PROSPERO (CRD42014007224)

## Strengths and Limitations

### Strengths include:

- A comprehensive review of the published literature from 1985-2013
- Literature search unrestricted by language or country
- Analysis of study designs resulted in novel strategy to limit bias and classify outcomes
- Three types of outcomes of dysbiosis applied to evidence-based studies of specific probiotic strains
- Author has over 40 years of research experience in the probiotic field

### Limitations include:

- Pooled clinical trials using different study populations
- Pooled probiotic doses and regimens
- Indirect evidence linking probiotic strains and dysbiosis
- Review done by sole author

## INTRODUCTION

The popularity of probiotics has expanded exponentially recently, but along with their increased use, debate rages on how probiotics should be regulated and whether probiotics should be considered as a medical food, drug or a food supplement. In the U.S., probiotics are typically available as dietary supplements and thus are limited to 'structure or function' health claims and, unlike prescription drugs, are not permitted to claim to 'treat' or 'cure' disease. In Europe and the United Kingdom, probiotics are allowed to have health or function claims. These claims are required to be supported by well-conducted human trials in the targeted population or in healthy volunteers, but the European Food Safety Authority (EFSA) has rejected >80% of claims submitted to them.<sup>1-3</sup> In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect.<sup>4</sup> One such functional claim made for probiotic products is they correct dysbiosis (or the disruption of bacterial and fungal species after antibiotics or other disruptive exposures) and thus may be beneficial to maintain health. Probiotics are active during this susceptible window from the time of the disruptive event to the time when normal microbiota is restored. A wide variety of mechanisms-of-action have been documented for probiotics (ranging from blocking pathogen attachment sites, destruction of the pathogen by bacteriocins or proteases that degrade toxins, to regulation of the immune system),<sup>5,6</sup> and while clinical evidence supports efficacy of some probiotic strains, the evidence linking these mechanisms-of-action to a specific health or function claims is not as clear.

A classic example of the consequence of dysbiosis is antibiotic-associated diarrhea (AAD).<sup>7,8</sup> While antibiotics may be effective in the elimination of pathogenic organisms, a common, unintended effect is the killing or inhibition of beneficial microbes due to shared susceptibility to the antibiotic. One of the many functions for normal microbiota is the ability to resist infection by pathogenic organisms, termed 'colonization resistance'.<sup>9,10</sup> The loss of a sub-population of the normal microbiota, for example, can lead to the loss of the ability to break down fibers and starches into absorbable short chain fatty acids, resulting in high level of undigested carbohydrates, which can trigger diarrhea.<sup>11</sup> Disruption of the normal microbiota has been shown to lead to higher rates of infections in other body systems other than the intestinal tract including the skin,<sup>12,13</sup> vagina,<sup>14,15</sup> respiratory tract,<sup>16,17</sup> and in the buccal cavity.<sup>18-20</sup>

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6 The major challenge to establishing a cause and effect for the improvement of dysbiosis by  
7 probiotics is a lack of a standard definition of 'normal' microbiota. There is substantial inter-  
8 individual variation of the species of microbes present at different body niches, which also varies  
9 by age, geographic area and health status of the host. In addition, a complete accounting of the  
10 microbiota is currently impossible, as there are no assays to detect all of  $>10^{13}$ - $10^{14}$  organisms in  
11 the intestines and standard microbial culturing methods miss 75-95% of these organisms.<sup>21,22</sup>  
12 The development of metagenomics (cataloguing individual and disease-specific bacterial gene  
13 profiles) and the creation of the international Human Microbiome Project ushered in a new era  
14 for our understanding of the complexity of these interactions within the body.<sup>23,24</sup> This paradigm  
15 shift from culturing to metagenomic analysis has expanded our ability to document shifts in  
16 microbial populations to an unparalleled degree, but the interpretation of these shifts continues to  
17 be under debate.<sup>25-28</sup> With the advent of these newer metagenomic tools, the role of probiotics in  
18 the restoration of normal microbiota is being re-visited.<sup>29</sup>  
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32 In light of new guidance documents and recommendations, the goal of this systematic review is  
33 to determine how claims for the restoration of the normal microbiota and the correction of  
34 dysbiosis have been studied using well-designed trials and which probiotic strains have  
35 evidence-based data to support these claims.  
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## 42 **METHODS**

### 43 **Study Objective**

44 To systematically review the literature to analyse the evidence for the claim probiotics can  
45 correct dysbiosis of the normal microbiota from randomised controlled trials.  
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### 52 **Search Strategy**

53 Search terms included: probiotics + health claims, restoring normal microbiota, dysbiosis,  
54 normal microbiota, pharmacokinetics, metagenomics, probiotics, dietary supplements,  
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3 randomized controlled trials, antibiotic associated diarrhea (AAD), *Clostridium difficile* infection  
4 (CDI), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), traveler's diarrhea  
5 (TD), eradication of *Helicobacter pylori*, bacterial vaginosis or vaginitis, treatment of acute  
6 pediatric diarrhea, and specific probiotic strains or products. Search strategies were broad-based  
7 initially, then narrowed to clinical trials with probiotics.  
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### 13 14 **Data Sources**

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16 PubMed (1985-2013), EMBASE (1985-2013), Cochrane Database of Systematic Reviews  
17 (1990-2013), CINAHL (1985-2013), AMED (1985-2013), and ISI Web of Science (2000-2013).  
18 Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled  
19 trials (<http://www.cochrane.org>), MetaRegister of Controlled Trials (<http://www.controlled->  
20 [trials.com/mrct](http://www.controlled-trials.com/mrct)) and National Institutes of Health (<http://www.clinicaltrials.gov>).  
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### 27 **Criteria for study selection and data extraction**

28 Abstracts of all citations were reviewed and rated for inclusion for randomized controlled trials  
29 of probiotic treatments. Full articles were retrieved if normal microbiota assays were mentioned.  
30 Non-English language trials were translated and included whenever possible. Exclusion criteria  
31 included pre-clinical studies (animal models or *in vitro* assays), safety or phase 2 studies,  
32 reviews, efficacy trials with no assays for normal microbiota species, metagenomic methods  
33 only, mechanism of action of normal microbiota or probiotic, cross-sectional surveys, case  
34 reports or case series, duplicate reports, or trials of unspecified types of probiotics. All  
35 pharmacokinetic studies in humans were reviewed, as abstracts often did not include normal  
36 microbiota assay data. Data extraction and the review process followed the PRISMA statement  
37 guidelines using a 27-item checklist and flow diagram.<sup>30</sup> A standardized data extraction form  
38 was used to collect data on the probiotic (strain type, daily dose, duration), type of controls  
39 (placebo, active or no treatment), study design (status of microbiota at baseline and follow-up  
40 times), type of microbiota assay (microbial culturing, molecular biomarkers, etc.), enrolled study  
41 population (adult vs. pediatric, healthy volunteers, disease condition), type and timing of  
42 disruptive agent (antibiotics, chemotherapy, etc.), study size and attrition, outcome assessment  
43 (efficacy and/or microbiota status at end of study, adverse events) and type of health claim.  
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## Outcomes and definitions

The primary outcome is the degree of microbiota correction or improvement by specific probiotic strain(s). The secondary outcome is the association between the degree of dysbiosis correction and the net efficacy found from randomized controlled trials of probiotic interventions. Dysbiosis is defined as an alteration or disruption of the normal microbiota (bacterial or fungal species) due to exposure of a disruptive factor (such as antibiotics, chronic disease, stress, medical procedures or medications, etc.). As there is no current standard definition of 'normal' microbiota, for this review, restoration of normal microbiota is defined as a return to the assayed microbial species or profile taken from a healthy individual (before a disruptive event has occurred). Included studies are required to have at least a pre-probiotic treatment assay and a post-probiotic treatment assay. A variety of microbial assays were available during the search period (1985-2013), including documentation of the microbiota by either microbial cultures, or metagenomic methods [16s rRNA-targeted probes using fluorescent *in situ* hybridization (FISH) or other polymerase chain reaction (PCR) technique]<sup>8,21,28,31</sup> or by indirect methods (Nugent scores).<sup>15</sup> Nugent scores (ranged 0-10) are used to diagnose bacterial vaginosis (scores  $\geq 7$ ) or normal vaginal microbiota (scores 0-3) based on the quantitated morphotypes of small gram negative rods (*G. vaginalis/Bacteroides* spp.) and curved gram negative rods (*Mobiluncus* spp.) from gram stains of vaginal discharge smears. Microbial assays of only the strain(s) contained in the probiotic product are considered as pharmacokinetic studies and were not included in the normal microbiota profiles.

**Models of dysbiosis.** To determine the impact on normal microbiota, only direct evidence of microbiota change (species, profiles, diversity indices, or diagnostic criteria) were included and indirect effects were excluded (changes in intestinal enzymes, immune system parameters or disease symptoms). The degree to which dysbiosis was improved is categorized into three levels: (1) recovery of the normal microbiota back to baseline levels; (2) alteration or improvement of the normal microbiota; and (3) no change in normal microbiota.

The literature contained three dysbiosis models: Model A (restoration of the normal microbiota), which assayed patients enrolled with a healthy, undisturbed microbiota and then assayed again after a disruptive event (such as antibiotic exposure) and probiotic therapy occurred; Model B (alteration of the microbiota) assayed patients with pre-existing disrupted microbiota (for

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3 example, pre-existing chronic disease or active disease) and then post-probiotic therapy; Model  
4 C (no dysbiosis) assayed volunteers with no disruptive event (before or during the clinical trial)  
5 at both pre-probiotic and post-probiotic times, as shown in Figure 1. 'Recovery' of the normal  
6 microbiota is defined as a restoration of the microbiota back to a normal healthy baseline.  
7 Recovery may be complete recovery (all assayed microbial levels returned to baseline) or  
8 incomplete recovery (partial recovery of some microbial strains, but not all returned to baseline  
9 levels). In studies enrolling subjects with dysbiosis at baseline (typically due to chronic  
10 diseases), it is not possible to show a restoration to normal microbiota levels because a normal,  
11 undisturbed microbiota was not present in these types of study subjects at the time of enrollment.  
12 Therefore, the strongest claim possible for Model B designs is for an 'alteration or improvement'  
13 of the microbiota. Only data from the probiotic-exposed subjects were analysed in this paper.  
14 Data from the control groups were used to confirm dysbiosis for subjects with chronic diseases  
15 or after a disruptive exposure, such as antibiotics or chemotherapy, unaffected by probiotic  
16 exposure.<sup>32-34</sup>  
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### 30 **Assessment of methodological strength and quality**

31 The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system  
32 for rating overall study quality will be used for each probiotic strain or type (single strains and  
33 mixtures of strains).<sup>35</sup> Recommendation for the support of the claim of each probiotic strain or  
34 mixture can be assessed by the overall strength of the evidence ["strong", many randomized  
35 controlled trials show significant recovery of the microbiota, or "moderate" only one randomized  
36 controlled trial; or "weak", only case series or reports, limited number of small trials, *etc.*].  
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44 Quality of the evidence is based on study design and graded as "high quality" (well-defined  
45 study design for determining restoration with normal microbiota, Model A), or "moderate  
46 quality" (disrupted microbiota at baseline, Model B), or "low quality" (no disruptive event  
47 occurred, Model C). Measurement of publication bias was not assessed for this review, as  
48 pooled outcome estimates of efficacy were not done, as typical in meta-analysis, but all studies  
49 with assays of microbiota were included to limit bias.  
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### 56 **Net efficacy rating**

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3 To determine if the ability to correct dysbiosis is associated with clinical efficacy, the published  
4 literature for randomized controlled trials (RCTs) or meta-analyses of probiotics for various  
5 disease indications, including antibiotic associated diarrhea (AAD),<sup>5,36,37</sup> *Clostridium difficile*  
6 infection (CDI),<sup>5,38</sup> inflammatory bowel disease (IBD),<sup>39</sup> irritable bowel syndrome (IBS),<sup>40</sup>  
7 traveler's diarrhea (TD),<sup>41</sup> eradication of *Helicobacter pylori* (Hp),<sup>36,37</sup> bacterial vaginosis (BV)  
8 or vaginitis,<sup>42</sup> and treatment of acute pediatric diarrhea was reviewed.<sup>43-45</sup> The net rank was  
9 calculated by subtracting the number of RCTs showing non-significant or equivalent efficacy  
10 from the number of RCTs having significant efficacies. The ranks were categorized as follows:  
11 ++,  $\geq 2$  net RCTs showing significant efficacy; +, net of one RCT showing significant efficacy; 0,  
12 equal number of RCTs showing significant and non-significant efficacy results and -,  $\geq 1$  net  
13 negative or non-significant RCTs. Probiotics with no RCTs were not ranked.  
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## 25 RESULTS

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27 A review of the literature from 1985-2013 found 353 articles that dealt with probiotic treatments  
28 and their potential effect on normal microbiota.  
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### 33 Excluded studies

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35 As shown in Figure 2, a total of 272 articles were excluded for the following reasons: reviews  
36 (n=116), probiotic efficacy studies with no data on normal microbiota assays (n=54), animal  
37 models of probiotics and changes in microbiota (n=38), metagenomic or microbiota methods  
38 only (n=17), studies on normal microbiota but with no use of probiotics (n=14), *in vitro* assays of  
39 microbiota (n=10), duplicative reports (n=2) or miscellaneous (n=21), which included probiotic  
40 mechanism of action studies, safety studies, duplicative reports, cross-sectional surveys and two  
41 with poorly described probiotic interventions.<sup>46,47</sup> A total of 81 full articles were reviewed which  
42 mentioned changes in normal microbiota or indicated a health claim for probiotics and effects on  
43 normal microbiota.  
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52 Probiotic pharmacokinetic studies (n=18) reporting concentrations of probiotic strains before and  
53 post-treatment, but did not assaying for other species of normal microbiota were excluded.  
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55 While several studies using this study design claim probiotics had an impact on normal  
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3 microbiota, type of data generated is pharmacokinetic behavior of the probiotics themselves and  
4 not the normal microbiota. Several studies stated that the normal microbiota was altered because  
5 an increase in various bacterial species was observed after the probiotics were given, but the  
6 species assayed were those contained in the probiotic product, so an increase is not unexpected.  
7 Pharmacokinetic studies have documented that probiotic strains taken orally can survive transit  
8 through the intestinal tract with recovery rates in feces ranging from <1% to 22%.<sup>48,49</sup> These  
9 pharmacokinetic studies were excluded from this analysis, as they did not assay other types of  
10 normal microbiota not found in the probiotic product.  
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### 20 **Included studies**

21 Of the 63 included clinical trials, five trials had multiple treatment arms, which resulted in a total  
22 of 68 treatment arms for analysis. Engelbrekton et al. tested a mixture of 5 probiotic strains in  
23 volunteers exposed to antibiotics and also tested a mixture of 4 probiotic strains in healthy  
24 volunteers with no antibiotic exposure.<sup>50</sup> Zoppi et al. had eight different treatment arms in his  
25 study, and probiotic arms were included in our analysis [*Saccharomyces boulardii* (*S. boulardii*)  
26 alone and *Lactobacillus* (*L.*) *rhamnosus* GG alone], a mixture of two probiotics (*L. acidophilus*  
27 and *Bifido. bifidum*) and a mixture of three probiotic strains (*L. acidophilus*, *L. rhamnosus* and  
28 *Bifido. bifidum*).<sup>51</sup> Orrhage et al. had two treatment arms (*Bifido. longum* alone and a mixture of  
29 *Bifido. longum* and *L. acidophilus*).<sup>52</sup> Larsen et al. tested two single probiotics (*Bifido. lactis* and  
30 *L. acidophilus*) in separate treatment arms.<sup>53</sup> Lidbeck et al. gave either enoxacin or clindamycin  
31 and randomized patients to either *L. acidophilus* or placebo.<sup>54</sup>  
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43 **Normal microbiota assay methods.** Of the 69 treatment arms that did normal microbiota assays,  
44 diverse methods were used to profile the microbiota. Many studies used only standard  
45 microbiological culture assays (37, 54%), while others (28, 40%) used techniques to detect non-  
46 cultivatable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16s rRNA  
47 sequencing) or other PCR techniques. Some studies (4, 6%) used an indirect measure of normal  
48 microbiota, using the Nugent score to diagnose bacterial vaginosis, which relies upon gram stain  
49 of the vaginal secretions, vaginal pH and symptoms to characterize if normal microbiota is  
50 present or absent.<sup>15</sup>  
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6 **Probiotic strains.** In the 69 treatment arms, most (36, 52%) used a single strain of probiotic,  
7 while 14 (20%) tested a mix of two probiotic strains and 19 (28%) tested a mix of three or more  
8 probiotic strains. The distribution of single versus multiple strain probiotics did not significant  
9 vary by the model of study design ( $X^2_2=2.3$ ,  $P=0.32$ ). Of the 15 restorative (Model A) study  
10 arms, 47% used a single strain of probiotic and 53% used multiple strains. Of the 25 treatment  
11 arms with disrupted microbiota at baseline (Model B), 44% used a single strain and 56% used  
12 multiple strains. Of the 29 study arms with undisturbed microbiota (Model C), 62% used a  
13 single strain and 38% used multiple strains.  
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### 23 **Normal microbiota restoration model (Model A)**

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25 Only 10 studies (with 15 treatment arms) using Model A to determine restoration of the  
26 microbiota were found (Table 1).<sup>32,34,50-52,54-58</sup> The type of enrolled subjects varied from healthy  
27 volunteers to children with untreated respiratory infections, to pediatric cancer patients. For  
28 subjects with acute infections or cancer, baseline assays were done prior to the disrupting agent  
29 (antibiotics or chemotherapy). The number of subjects given probiotics averaged 20/study and  
30 ranged from 5 to 83. In 93%, the disruptive factor was antibiotic exposure and in one study,  
31 chemotherapy caused the microbiota disruption. Only 8 (53%) of the study arms did an assay  
32 during a 1-8 week follow-up period after the probiotic was discontinued.  
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43 Analysis of the probiotic strain(s) separately found only two probiotic products with more than  
44 one randomized controlled trial. The probiotic mix of *L. acidophilus* and *Bifido. bifidum* showed  
45 a complete restoration in one study, but only a partial recovery in the other. (Strength: strong,  
46 Quality: high). The probiotic mix of *L. acidophilus* (2 strains) with *Bifido. bifidum* and *Bifido.*  
47 *animalis* showed complete restoration in one study, but only a partial recovery in the other.  
48 (Strength: strong, Quality: high). Five other probiotic products with only one supporting clinical  
49 trial showed microbiota restoration (*Bifido. longum*, *Clostridium butyricum*, *L. acidophilus*, mix  
50 of *L. acidophilus* with *L. paracasei* and *Bifido. lactis*, and the mix of *L. acidophilus* with *L.*  
51 *paracasei* and *Bifido. bifidum* and two strains of *Bifido. lactis*). (Strength: moderate, Quality:  
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3 high). Three probiotic products with one supporting clinical trial showed partial restoration (*S.*  
4 *boulevardii*, *L. rhamnosus* GG, mix of *L. rhamnosus* with *L. bifidus* and *L. acidophilus*), (Strength:  
5 moderate, Quality: high). Only two probiotic products using Model A showed no change in the  
6 microbiota (*Bifido. breve* and a mix of *L. acidophilus* and *Bifido. longum*). (Strength: moderate,  
7 Quality: high). In summary, 10 of 12 (83%) of the probiotic products showed complete or partial  
8 restoration of the normal microbiota.  
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18 Of the 11 probiotic products with claims of 'restores or improves normal microbiota', 10 (91%)  
19 were supported by this review, but only seven showed complete restoration and five had partial  
20 restoration of the microbiota (Table 1). The mixture of *L. acidophilus* and *Bifido. longum* did  
21 not show any changes in the microbiota. Wada et al. claimed *Bifido. breve* 'enhanced intestinal  
22 anaerobes', but this was only compared to the placebo group.<sup>32</sup> Their data showed chemotherapy  
23 is a disruptive event, resulting in more Enterobacteria in the intestine in the placebo group, but  
24 there were no significant differences seen by the end of the 8 week follow-up in either the  
25 probiotic or the placebo group compared to baseline microbiota levels.  
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### 35 **Disrupted normal microbiota at baseline studies (Model B)**

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38 Twenty-four studies (with 25 treatment arms) used Model B that enrolled subjects with a pre-  
39 existing disrupted microbiota related to ongoing disease or conditions (Table 2).<sup>33,53,59-80</sup> The  
40 number of subjects given probiotics averaged  $23 \pm 16$ /study and ranged from 7-83 participants.  
41 The types of pre-existing factors that disrupted the microbiota included atopic dermatitis  
42 patients, allergies, cirrhosis, bacterial vaginosis, irritable bowel syndrome, inflammatory bowel  
43 disease (ulcerative colitis and pouchitis), idiopathic diarrhea, enteral feeding, short-bowel  
44 syndrome and colon cancer. Only 10 (40%) of the study arms did an assay during the post-  
45 probiotic follow-up period.  
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55 Three of the probiotics had multiple clinical trials to support the claim of an improvement in the  
56 microbiota due to the probiotic. *S. boulevardii* was used in two trials either with enteral fed  
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3 patients or patients with active diarrhea and found an improvement in the habitual microbiota in  
4 the patients with active diarrhea<sup>66</sup>, but only showed indirect evidence of short-chain fatty acid  
5 changes in the other study.<sup>65</sup> (Strength: strong, Quality: moderate) A mix of four probiotic  
6 strains (2 strains of *L. rhamnosus*, *P. freudenreichii* + *Bifido. breve*) showed improved  
7 microbiota in two clinical trials.<sup>74,75</sup> (Strength: strong, Quality: moderate) Of four clinical trials  
8 testing a mixture of seven probiotic strains, two showed no significant change in microbiota<sup>77,78</sup>,  
9 one showed more anaerobes post-probiotic treatment<sup>79</sup> and one found a reduction in *Bacteroides*  
10 species.<sup>80</sup> (Strength: strong, Quality: moderate) Three clinical trials determined there were no  
11 significant changes due to *L. plantarum* 299v.<sup>62-64</sup> (Strength: strong, Quality: moderate). Of those  
12 probiotics with only one supporting clinical trial (Strength: moderate, Quality: moderate), two  
13 single probiotic strains (*E. coli* Nissle and *L. casei rhamnosus*) and five different mixtures of  
14 probiotic strains support the claim that the probiotic alters the microbiota (Table 2). In summary,  
15 10 of 18 (56%) probiotic products altered or improved microbiota in individuals with pre-  
16 existing disease.  
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32 Of the 25 treatment arms, the paper's claim was confirmed in 14 (56%) of the studies. There was  
33 no significant change in the microbiota due to the probiotic in nine treatment arms and only an  
34 alteration of the microbiota in five others (Table 2). Our review disagreed with the claimed  
35 outcomes in 11 (46%) of the other treatment arms. In seven treatment arms, it was claimed the  
36 tested probiotic 'restored normal microbiota', but it is uncertain how this conclusion was reached,  
37 since there was no time when a normal undisrupted microbiota was present. Of the seven studies  
38 that claimed their probiotic 'restored' normal microbiota, our analysis determined none were  
39 capable of documenting restoration, but it is confirmed probiotics improved or altered the  
40 microbiota in these studies. Four studies claimed the probiotic 'altered or improved' normal  
41 microbiota, but this review found no significant differences when post-probiotic and baseline  
42 assays were compared for the probiotic groups. Girard-Pipau et al. concluded that *S. boulardii*  
43 'altered normal flora' because more gram positive anaerobes were seen in the probiotic group  
44 compared to the controls and an increase in three short-chain fatty acids were observed in the *S.*  
45 *boulardii* group.<sup>65</sup> However, when the analysis is restricted to trends observed in the probiotic  
46 group only, no significant differences were observed in pre-probiotic versus post-probiotic  
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3 microbiota profiles. Venturi et al. concluded that the mix of seven probiotic strains enhanced the  
4 concentration of some beneficial strains in the intestines.<sup>77</sup> However, the only strains having a  
5 significant increase were those contained in the probiotic mix, and not specifically normal  
6 microbiota of the host. As this study did not have an undisturbed microbiota baseline, the  
7 increased numbers of Lactobacilli and Bifidobacteria may not have reflected their normal levels.  
8 Van der Aa et al. claimed that *Bifido. breve* 'successfully modulates the intestinal flora', but no  
9 significant changes were observed in the probiotic group when comparing the baseline to the  
10 post-probiotic levels.<sup>59</sup> Odamaki et al. did show an increase in *Faecalibacterium* ssp. and  
11 *Bacteroides fragilis* ssp. at the end of *Bifido. longum* BB536 treatment, but the same increase  
12 was also observed in the placebo group.<sup>33</sup>  
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### 25 **Undisrupted normal microbiota studies (Model C)**

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27 Twenty nine trials enrolled healthy adults who had no disruptive factor present during the study  
28 (either no antibiotic or no medication exposure or presence of acute or chronic disease) that  
29 might impact normal microbiota, as shown in Table 3.<sup>14,49,50,81-106</sup> The average number of  
30 subjects given probiotics was 23/study and ranged from 7 to 160/study. Of the 29 study arms,  
31 assays were taken during a follow-up period in only 52%. Fujiwara et al. cultured seven healthy  
32 volunteers and found Enterobacteriaceae and Clostridial species post-*Bifido. longum* was  
33 reduced by 10<sup>1</sup>/g compared to baseline (P<0.03), but no other changes in the microbiota were  
34 detected.<sup>84</sup> Karlsson et al. found a significant increase in intestinal diversity in nine male  
35 volunteers with atherosclerosis given *L. plantarum* 299v, but because terminal restriction  
36 fragment length polymorphism assays were used instead of cultures for bacterial species, the  
37 specific changes in the microbiota species could not be determined.<sup>94</sup> Yang and Sheu cultured 63  
38 children (55% with *H. pylori*) given a yogurt with *L. acidophilus* and *Bifido. lactis* but only  
39 found a decrease in *E. coli* counts in the *H. pylori* negative children sub-group, no significant  
40 changes in normal microbiota was found in the *H. pylori* positive children.<sup>100</sup> Kubota et al.  
41 assayed 29 subjects with Japanese cedar pollen allergy and found milk fermented with *L.*  
42 *rhamnosus* GG and *L. gasseri* TMC0356 suppressed microbiota changes (less intestinal profile  
43 changes), but could not determine specific bacterial species changes due to the type of assay used  
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3 (FISH and TRFLP).<sup>103</sup> In summary, only 4 of 19 (21%) probiotic products altered microbiota in  
4 healthy individuals who had no disruptive event.  
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10 Of the seven studies that claimed their probiotic(s) 'restored or altered' the normal microbiota,  
11 only four claims were confirmed. Sierra et al. claimed *L. salivarius* given to 20 healthy adults  
12 'improved gut microbiota', but only increased levels of Lactobacilli were found and no other  
13 changes in normal microbiota species were detected. The only other evidence was indirect from  
14 changes observed in immune parameters.<sup>96</sup> He et al. claimed a mixture of *Bifido. longum* and  
15 *Bifido. animalis* 'modified' microbiota, but changes were seen only during the yogurt  
16 administration and not after the one week follow-up period.<sup>99</sup> Vitali et al. claimed that the  
17 mixture of four Lactobacilli strains and three Bifidobacteria strains 'modulated vaginal  
18 microbiota', but the only significant changes were due to an increase in the bacterial species  
19 contained in the probiotic mixture.<sup>14</sup>  
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31 Of the probiotics supported by multiple clinical trials (*Bifido. animalis*, *Bifido. longum*, *L. casei*,  
32 *L. plantarum* 299v, the mixture of *Bifido. animalis* and *Bifido. lactis*), 13 of the trials (87%)  
33 support there is no significant change in normal microbiota if the microbiota is not disrupted.  
34 [Strength: strong, Quality: low]  
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#### 42 **Association of clinical efficacy and normal microbiota restoration**

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44 Few studies concurrently compared clinical efficacy and the ability to restore or improve normal  
45 microbiota after dysbiosis. A synthesis of the literature of RCT for eight common disease  
46 indications was performed and the overall net strength was ranked. Probiotics with the ability to  
47 restore normal microbiota were frequently supported by RCTs for efficacy, as shown in Table 4.  
48 Of the 10 probiotics with evidence for restoration, 7 (70%) also had at least one RCT testing for  
49 at least one of the eight diseases, while 30% did not have any supportive RCTs for efficacy. Of  
50 the 7 probiotics with associated RCTs, only two probiotics (*S. boulardii* and *L. acidophilus*) have  
51 strong evidence for efficacy across most of the disease indications, while five probiotics with the  
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3 ability to restore the microbiota had weak or no evidence of efficacy. For example, *S. boulardii*,  
4 which has studies supporting restoration, has strong evidence for clinical efficacy for AAD  
5 (ranked ++: 11 RCTs had significant results and 6 had non-significant results), CDI (ranked ++:  
6 had two RCTs with significant results), IBD (ranked ++: had two RCTs with significant results),  
7 IBS (ranked 0: had one RCT with significant efficacy and one RCT with non-significant results),  
8 TD (ranked +: 3 RCTs with significant efficacy and 2 with non-significant efficacy), *H. pylori*  
9 eradication (ranked -: 2 RCTs with significant results and 4 with non-significant results) and no  
10 studies for BV. *L. acidophilus*, which partially restored the microbiota in a study, is associated  
11 with clinical efficacy for AAD, IBS and BV, but not for TD or eradication of *H. pylori* and  
12 treatment of acute pediatric diarrhea (ranked ++: had 19 RCTs with significant protection and  
13 five with non-significant results). In contrast, *L. rhamnosus GG*, another probiotic capable of  
14 restoring microbiota, is often cited in meta-analysis as having significant efficacy for AAD. Our  
15 results of an updated review of the literature indicate a net weak evidence rating for clinical  
16 efficacy across all disease indications: AAD (ranked -: 3 RCTs had significant results and 6 had  
17 non-significant results), CDI (ranked -: two RCTs with non-significant results), IBD (ranked -:  
18 one RCT with non-significant results), IBS (ranked 0: 2 RCTs with significant efficacy and two  
19 RCTs with non-significant results), TD (ranked 0: one RCT with significant efficacy and one  
20 with non-significant efficacy), *H. pylori* eradication (ranked -: 3 RCTs with non-significant  
21 results), no RCTs for BV and treatment of acute pediatric diarrhea (ranked ++: 10 RCTs with  
22 significant efficacy and one with non-significant findings).  
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42 Efficacy trials were not done as frequently for probiotics shown to only have the ability to alter  
43 or improve, but not restore, the microbiota after dysbiosis. Of nine probiotics that can alter the  
44 microbiota, 6 (67%) have supporting RCTs for at least one disease, but the diversity of  
45 investigated diseases was more limited. *L. casei* had moderate net strength for AAD and  
46 bacterial vaginosis, but was neutral for the ability to eradicate *H. pylori* and other disease  
47 indications were not tested in RCTs with *L. casei*. The probiotic mixture of *L. reuteri* and *L.*  
48 *fermentum* has strong evidence for bacterial vaginosis, but not for any other disease indications  
49 listed in Table 4.  
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3 Of the eight probiotics not capable of altering or restoring normal microbiota, only *L. plantarum*  
4 299v had RCTs for AAD and IBS, both with net negative or weak strength of clinical efficacy.  
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6 *Bifido. lactis* and the mixture of *L. rhamnosus* and *L. reuteri* had net neutral rankings for efficacy  
7 for the treatment of acute pediatric diarrhea. The other four probiotic products with no effect on  
8 normal microbiota lacked any RCTs for clinical efficacy. Studies with *B. clausii* did not assay  
9 for normal microbiota and had non-significant trial results for *H. pylori* eradication and the  
10 treatment of pediatric diarrhea.  
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18 Of the six probiotics with only pharmacokinetic data on the probiotic itself and no other  
19 investigation of other normal microbiota strains, five had RCTs showing varying net efficacies  
20 for different disease indications, as shown in Table 4.  
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25 Six popular probiotics (*Bacillus clausii*, *Bifido. infantis*, *L. reuteri*, *L. acidophilus* + *L. helveticus*,  
26 *L. acidophilus* + *L. casei* and *L. acidophilus* + *Bifido. animalis*) have only clinical efficacy  
27 RCTs, but have not published studies investigating their role in restoring or improving the  
28 normal microbiota.  
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## 34 Discussion

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36 Developing and evaluating health or function claims for probiotics is an important issue and is  
37 now identified as a priority for research by several international organizations, including the  
38 World Gastroenterology Organization<sup>107</sup> and the American Society for Nutrition.<sup>2</sup> The U.S. Food  
39 and Drug Administration has struggled with appropriate evidence-based health claims for  
40 probiotic products and currently recommends the use of structure/function claims, such as  
41 "maintains bowel regularity", but the claim for restoring normal microbiota is still under  
42 debate.<sup>108</sup> The European Food Safety Authority (EFSA) provides guidance materials that  
43 recommend health or function claims for probiotics should have beneficial physiological effects  
44 and have appropriate scientific trials to substantiate the health claims.<sup>3</sup> Acceptable claims for  
45 intestinal health may include functional claims (improved transit time, softer stool consistency,  
46 reduction in gastrointestinal discomfort, defense against pathogens). As it is currently not  
47 possible to define a standard normal microbiota profile, the EFSA recommends functional claims  
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3 for the restoration of normal microbiota should document a recovery of healthy microbiota and  
4 be accompanied by a beneficial physiological or clinical outcome.<sup>3</sup> In addition, because the  
5 efficacy and mechanisms are strain-specific and may vary by probiotic strain, the evidence must  
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7 be analyzed for each probiotic product individually.<sup>5,6,9,109, 110-112</sup>  
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12 An underappreciated finding was the influence that study design and study populations have on  
13 the interpretation of study outcomes. In the literature, five different types of study designs are  
14 commonly found relating to probiotics. The most common study type is a randomized controlled  
15 trial testing the efficacy and safety outcomes in patients, but these trials did not typically  
16 document the impact of the probiotic on the normal microbiota. The second most common type  
17 of study design is pharmacokinetic studies (documenting recovery of oral dose of probiotic or  
18 increase in probiotic strains post-treatment compared to pre-treatment or clearance of the  
19 probiotic). Even though these kinetic studies did not assay for non-probiotic strains, some  
20 extrapolated their results and concluded some effect or improvement of the normal microbiota  
21 was observed by their probiotic.<sup>19,111</sup> These two first types of study designs do not support  
22 evidence-based conclusions for the restoration or alteration of the normal microbiota and were  
23 excluded from this review.  
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34 Three types of study designs are appropriate for the study of dysbiosis. The first type of study  
35 design had normal microbiota assayed at least twice (at baseline, which was before exposure to a  
36 disruptive event or probiotics and then again during or post-probiotic treatment) to show actual  
37 recovery of assayed normal microbiota back to healthy baseline levels. The second type of study  
38 design started with inappropriate baselines (baseline samples taken after normal microbiota had  
39 been disrupted by chronic disease). For patients with established chronic diseases, there is no  
40 “normal microbiota” baseline in either the probiotic or the control group. Even if baselines are  
41 taken during remission, the microbiota may still be impacted by chronic disease or acute  
42 diarrhea. Studies of probiotics in chronic diseases or acute disease typically report on ‘pre-  
43 probiotic treatment’ and ‘post-probiotic treatment’ and may show significant shifts in microbial  
44 species, but it is uncertain if this reflects a true re-establishment of normal microbiota profiles.  
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46 The third type of study design enrolled healthy volunteers, who were not challenged with  
47 antibiotics (so no normal microbiota disruption occurred), and show only the effect of probiotics  
48 on a healthy microbiota (typically mild or no effects). Control groups were not required for our  
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3 assessment of the impact of probiotics on microbiota, but control groups can document the  
4 degree normal microbiota is disrupted by inciting agents (antibiotic, disease onset, etc.).  
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10 Five single strain probiotics (*Bifido. longum*, *Clost. butyricum*, *L. acidophilus*, *L. rhamnosus* and  
11 *S. boulardii*) and five probiotic mixtures [(*L. acidophilus* + *Bifido. bifidum*), (*L. rhamnosus* + *L.*  
12 *bifidus* + *L. acidophilus*), (*L. acidophilus* + *L. paracasei* + *Bifido. lactis*), (*L. acidophilus*, 2  
13 strains, *Bifido. bifidum*, *Bifido. animalis*) and (*L. acidophilus* + *L. paracasei* + *Bifido. bifidum*  
14 + 2 strains of *Bifido. lactis*)] documented either complete or partial recovery of normal  
15 microbiota (Model A). Only two probiotic mixtures [(2 strain mixture: *L. acidophilus* + *Bifido.*  
16 *bifidum*) and (4 strain mixture: *L. acidophilus*, 2 strains, *Bifido. bifidum*, *Bifido. animalis*)] were  
17 supported by a confirmatory study. Evidence that probiotics may alter or improve normal  
18 microbiota (Model B) was found for three single strain probiotics (*E. coli* Nissle, *S. boulardii*  
19 and *L. casei rhamnosus*) and seven mixtures of 2-7 probiotic strains. Of these ten probiotics  
20 finding alteration of the microbiota, only three had multiple trials [*S. boulardii*, and a four strain  
21 mixture (2 strains of *L. rhamnosus* + *P. freudenreichii* + *Bifido. breve*), and a seven strain  
22 mixture (4 Lactobacilli and 3 Bifidobacteria strains)], but only one had consistent results  
23 showing improvements in the microbiota.<sup>74,75</sup> Clearly, more than one study is needed to confirm  
24 the impact of a probiotic on the normal microbiota. Of the 19 probiotic strains (or mixtures)  
25 studied in healthy volunteers who were not exposed to disruptive factors (Model C), no change  
26 in the normal microbiota was observed for 79%, indicating the robustness of the microbiota.  
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44 Improvement in the normal microbiota by specific probiotic strains seemed to be associated with  
45 better clinical endpoints. Within eight common diseases typically treated with probiotics, more  
46 trials with significant efficacy were associated with probiotic strains shown to restore the normal  
47 microbiota, and only one trial with significant efficacy was found for probiotics that did not alter  
48 the microbiota. However, few probiotics had efficacy trials for all eight diseases and many did  
49 not have any efficacy trials.  
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Some probiotics which have published efficacy trials for various diseases did not have studies investigating the effect of the probiotic on normal microbiota: *Bacillus clausii*, *Bifido. infantis*, *L. brevis*, *L. reuteri*, mix of 2 strains (*L. acidophilus* + *L. helveticus*), mix of 2 strains (*L. acidophilus* + *L. casei*) or (*L. acidophilus* + *Bifido. animalis*), mix of 4 strains [*L. rhamnosus* (2 strains), *Propionibacterium freudenreichii* + *Bifido. animalis*] and mix of 7 strains (*L. sporogens*, *L. bifidum*, *L. bulgaricus*, *L. thermophilus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*).

### Comparison of results with other studies

Other reviews in the literature of claims for probiotics relating to changes in the normal microbiota have focused on the broad issues of regulatory standardization of health or function claims, the use of proper study designs and the challenge of defining biomarkers for a 'healthy microbiota'.<sup>3,29,112</sup> Donovan et al. recommends that health claims for probiotics be supported by well-conducted human trials in the targeted population.<sup>2</sup> These reviews also recommend that gut biomarkers need to be correlated with clinical endpoints, however none of these reviews attempted to do so.<sup>29,112</sup> No prior review has attempted to analyze the association between probiotic strains and their impact on normal microbiota by stratifying on the quality of study design.<sup>111</sup> This review addressed these concerns by analyzing probiotic strains by the quality of the study design and only including trials that assessed the normal microbiota (either by microbial culturing or molecular strain biomarkers) and assessed the degree of dysbiosis improvement with clinical outcomes for each probiotic strain.

### Opportunities for future research

Most of the studies (80%) using Model A to document restoration of the normal microbiota only used microbiologic culturing techniques, which can only detect those organisms that grow in culture. Use of the more advanced molecular metagenomic techniques have found that culturing alone misses up to 95% of these organisms.<sup>21,22</sup> The use of the metagenomic techniques was more common in the studies using Model B (48%) and Model C (45%) study designs, which only addresses potential alteration of the microbiota. Characterization of the microbiota is a

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3 complex issue and a comprehensive accounting of all the bacterial and fungal strains in the body  
4 is beyond our current capabilities. Therefore, any studies of changes to the microbiota are  
5 incomplete at best, but general trends in bacterial phylotypes can be documented using DNA  
6 probes and metagenomic techniques. Differential detection bias may be present due to the  
7 variety of assays used in these studies and should be accounted for in future studies.  
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16 Another suggestion for future studies is to include an appropriate follow-up time period post-  
17 probiotic administration. Fewer than half of the reviewed trials did assays for normal microbiota  
18 during an appropriate follow-up period. As it has been shown that recovery from a disrupting  
19 factor can be prolonged (typically eight weeks),<sup>7,8</sup> and studies that failed to find microbiota  
20 recovery might have detected a return to normal baseline levels if a sufficiently long time was  
21 given for the recovery to have occurred. Future studies should strive to allow time for the  
22 restoration of the normal microbiota to occur.  
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31 As the effects of probiotics are strain-specific, and many studies typically only report the genus  
32 and species of the tested probiotic, future reports should include a complete description of the  
33 probiotic to the strain level.<sup>5,112</sup>  
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### 39 **Strengths and weaknesses**

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42 The strengths of this review included the completeness of the search strategy, which reviewed  
43 multiple citation databases, trial registries and author searches, use of established PRISMA  
44 protocols for reviews and the use of an outcome classification scheme for different degrees of  
45 assessment for microbial recovery. This analysis controlled the confounding effects of different  
46 study populations and study designs present in the literature. Pharmacokinetic studies of just the  
47 probiotic strain(s) itself were excluded and only trials that assayed other species found in the  
48 microbiota were included. By applying a standard definition for 'restoring' versus 'improving'  
49 normal microbiota, it is possible to distinguish significant differences by the type of study  
50 designs used and differential effects of the different probiotic strains. Limitations of this review  
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3 include pooling trials from different populations (adult versus pediatric) and different probiotic  
4 doses and regimens used. Incomplete retrieval of all studies assessing the effect that probiotics  
5 have on human microbiota is also a potential limitation of any literature search. Another  
6 limitation is that dysbiosis improvement and clinical efficacy for probiotic strains is also  
7 indirectly associated, no direct cause and effect relationship was possible with the types of  
8 studies done. Another limitation is the current lack of a standard definition of what comprises a  
9 'normal microbiota'. The constituents of the microbiota vary by individual, by age, geographic  
10 location and health status of the host. Current microbiologic techniques are improving, but can  
11 not detect all species present in the host.  
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## 22 **Conclusion**

23 The challenges in recommending a specific probiotic to patients who need to restore or improve  
24 their normal microbiota after a disrupting event occurs is two-fold: one is the diversity of  
25 probiotic products available and second is the varying strength of evidence provided by clinical  
26 trials using different outcome measures and study designs. By grouping studies into three groups  
27 that result in three different degrees of probiotic effect (restoration, improvement or no change),  
28 an overview of the body of evidence is possible. By comparing the strength of the clinical  
29 evidence for common diseases by the degree to which the probiotics could impact the restoration  
30 of the normal microbiota, it became obvious that those probiotics with a greater ability to restore  
31 the microbiota are associated with the strongest strength of clinical efficacy. While this evidence  
32 only indirectly links clinical efficacy with the ability to restore the microbiota, the overall review  
33 of the evidence shows this is an important mechanism of action for probiotics. What becomes  
34 obvious is that more studies are required to conclude which probiotic strains have a beneficial  
35 impact on the normal microbiota, as most strains have only a single clinical trial and many  
36 probiotic products overstate the strength of their claim to restore normal microbiota. These types  
37 of issues should be considered for health care policy makers and researchers for future studies  
38 and for creating guidelines for health/function claims.  
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**Table 1. Model A:** Evidence-based data for restoration of normal microbiota (NM) for 12 probiotics from 10 studies (15 treatment arms).

Probiotic*	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment (wks)	Claims stated in papers	Evidence-based claim
<i>Bifido. breve</i>	Wada 2010 <sup>32</sup>	19	FISH	pediatric cancer patients	chemotherapy	8	enhances anaerobes	no change
<i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	restores	restores
<i>Clost. butyricum</i> MIYAIRI	Seki 2003 <sup>34</sup>	83	culture	pediatric respiratory or GI infections	antibiotics	0	restores	restores
<i>L. acidophilus</i> NCFB1748	Lidbeck 1988 <sup>54</sup>	5	culture	healthy volunteers	enoxacin or	1	restores only in enoxacin	restores only in enoxacin,
		5	culture	volunteers	clindamycin	1	no change	no change in clindamycin
<i>L. rhamnosus</i> GG	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>S. boulardii</i> lyo	Zoppi 2001 <sup>51</sup>	6	culture	pediatric respiratory infections	ceftriaxone	0	improves	partially restores
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	Black 1991, <sup>55</sup> Zoppi 2001 <sup>51</sup>	10,	culture,	healthy volunteers,	ampicillin,	2,	recovers more rapidly,	restores,
		7	culture	pediatric respiratory	ceftriaxone	0	less change	partially restores
<i>L. acidophilus</i> 1748 + <i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	no change	no change
<i>L. rhamnosus</i> + <i>L. bifidus</i> + <i>L. acidophilus</i>	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido lactis</i> Bb12	Jernberg 2005 <sup>56</sup>	4	culture PCR TRFLP	healthy volunteers	clindamycin	2	restores	restores
<i>L. acidophilus</i> CUL60+ <i>L. acidophilus</i> CUL21 + <i>Bifido. bifidum</i> CUL17 + <i>Bifido animalis lactis</i>	Madden 2005, <sup>57</sup> Plummer 2005 <sup>58</sup>	15,	culture,	<i>H. pylori</i> +,	amoxicillin + metronidazole,	2,	restores,	restores,
		76	culture	<i>H. pylori</i> +	amoxicillin + clarithromycin	2	restores more rapidly	partially restores
<i>L. acidophilus</i> NCFM + <i>L. paracasei</i> Lpc-37 + <i>Bifido. bifidum</i> Bb02+ <i>Bifido. lactis</i> Bi-04 + <i>Bifido. lactis</i> Bi-07	Engelbrektsen 2006 <sup>50</sup>	20	culture PCR TRFLP	healthy volunteers	augmentin	2	restores	restores

\*including strain (when reported)

**Table 2. Model B:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 18 probiotics from 24 studies (25 treatment arms) with disturbed microbiota at baseline.

Probiotic*	Reference	No. treated with probiotic	Type(s) of assay for NM	Pre-existing disrupting factor**	Follow-up time	Claims stated in papers	Evidence-based claim	Type of change found in NM
<i>Bifido. breve</i> M-16V	Van der Aa 2010 <sup>59</sup>	46	FISH	Atopic dermatitis	0	modulates NF	no change	--
<i>Bifido. lactis</i> Bi-07	Larsen 2011 <sup>53</sup>	17	PCR	Atopic dermatitis	0	no change	no change	--
<i>Bifido. longum</i> BB536	Odamaki 2007 <sup>33</sup>	22	TRFLP PCR	Cedar pollen allergy	4 wk	maintains NF	no change	--
<i>E. coli</i> Nissle	Lata 2007 <sup>60</sup>	22	culture	liver cirrhosis	0	restores	improves	more Bifido. & Lacto.
<i>L. acidophilus</i> 700396	Larsen 2011 <sup>53</sup>	17	PCR	atopic dermatitis	0	no change	no change	--
<i>L. casei rhamnosus</i> Lcr35	Petricevic 2008 <sup>61</sup>	83	Nugent scores	bacterial vaginosis	4 wk	restores	improves	improved Nugent scores
<i>L. plantarum</i> 299v	Nobaek 2000, <sup>62</sup>	25,	culture,	IBS,	4 wk,	no change,	no change,	--
	Klarin 2005, <sup>63</sup>	17,	culture,	enterally-fed,	0,	no change,	no change,	
	Klarin 2008 <sup>64</sup>	22	culture	antibiotics	0	no change,	no change,	
<i>S. boulardii</i> Iyo	Girard 2002, <sup>65</sup> Swidsinski 2008 <sup>66</sup>	10,	culture,	enterally-fed,	9 d,	alters NF,	no change,	--
		20	FISH	active diarrhea	3 wk	improves	improves	more 'habitual microbiota'
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2001 <sup>67</sup>	33	Nugent scores	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2003 <sup>68</sup>	31	Nugent scores and culture	bacterial vaginosis	30 d	restores	improves	improved Nugent scores
<i>L. plantarum</i> 8PA3 + <i>Bifido bifidum</i>	Kirpich 2008 <sup>69</sup>	32	culture	colon cancer	0	restores	improves	more <i>E. coli</i> and <i>Enterococci</i>
<i>L. rhamnosus</i> GR1 + <i>L. reuteri</i> RC14	Hummelen 2010 <sup>70</sup>	23	Nugent score	bacterial vaginosis	0	no change	no change	--
<i>L. casei</i> Shirota+ <i>Bifido breve</i> BBG01	Uchida 2007 <sup>71</sup>	4	culture	short bowel syndrome	0	no change	no change	--
<i>L. brevis</i> CD2 + <i>L. salivaris</i> FV2 + <i>L. plantarum</i> FV9	Mastromarino 2009 <sup>72</sup>	19	Nugent score	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores

<i>L. paracasei</i> Lpc37 + <i>L. acidophilus</i> 74-2 + <i>Bifido. animalis</i> DGCC420	Roessler 2012 <sup>73</sup>	30	PCR	atopic dermatitis	0	no change	no change	--
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii shermanii</i> JS + <i>Bifido. breve</i> Bb99	Kajander 2005, <sup>74</sup>	41,	PCR,	IBS,	0,	restores,	improves,	Improved similarity index
	Lyra 2010 <sup>75</sup>	22	PCR	IBS	0	alters	alters	More Clostridia and Rumino-coccus
<i>L. acidophilus</i> 4356 + <i>L. plantarum</i> 14917 + <i>L. rhamnosus</i> 7469 + <i>Bifido. bifidum</i> 2952	Wong 2013 <sup>76</sup>	7	PCR	liver disease	0	improves	alters	Less Firmicutes, more Bacteroidetes
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp. bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Venturi 1999, <sup>77</sup>	20,	culture	ulcerative colitis,	15 d,	enhances,	no change,	--
	Brigidi 2001, <sup>78</sup>	10,	culture & PCR	IBS,	10 d,	no change,	no change,	--
	Kuhbacher 2006 <sup>79</sup>	10	FISH	pouchitis	0	altered richness	altered	More anaerobes
	Ng 2013 <sup>80</sup>	10	PCR	IBS	0	modulates	altered	Less Bacteroides

\*including strain (when reported)

\*\*disruption of normal microbiota at baseline shown by significant differences compared to control (non-diseased) population.

**Table 3. Model C:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 19 probiotics in healthy volunteers enrolled in 29 studies (29 treatment arms) in studies with no disruptive exposures.

Probiotic	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment	Claims stated in papers	Evidence-based claim
<i>Bifido. animalis lactis</i> DN173010	Rochet 2008, <sup>49</sup> Oswari 2013 <sup>81</sup>	12, 160	FISH PCR	healthy volunteers	none, none	10 d, 6 mon	no change, no change	no change, no change
<i>Bifido. bifidum</i>	Langhendries 1995 <sup>82</sup>	20	culture	healthy volunteers	none	0	no change	no change
<i>Bifido. longum</i>	Benno 1992, <sup>83</sup> Fujiwara 2001, <sup>84</sup> Harmsen 2002 <sup>85</sup>	5, 7, 14	culture, culture, FISH	healthy volunteers	none, none, none	0, 30 d, 0	no change, alters, no change	no change, alters, no change
<i>L. casei</i> ND114001	Guerin 1998, <sup>86</sup> Rochet 2006, <sup>87</sup> Rochet 2008 <sup>88</sup>	12, 12, 7	culture, FISH, FISH	healthy volunteers	none, none, none	1 wk, 10 d, 0	no change, no change, no change	no change, no change, no change
<i>L. johnsonii</i> La1	Brunser 2006 <sup>89</sup>	32	culture & FISH	healthy volunteers	none	2 wk	no claim	no change
<i>L. plantarum</i> 299v	Goossens 2003, <sup>90</sup> Goossens 2005, <sup>91</sup> Goossens 2006, <sup>92</sup> Berggren 2003, <sup>93</sup> Karlsson 2010 <sup>94</sup>	11, 32, 15, 33, 9	culture, culture, culture, TRFLP	healthy, healthy, colonic polyps, healthy, atherosclerosis	none, none, none, none, none	3 wk, 4 wk, 0, 0, 0	no change, no change, no change, no change, alters	no change, no change, no change, no change, alters
<i>L. rhamnosus</i> GG	Gueimonde 2006 <sup>95</sup>	29	PCR	healthy volunteers	none	0	no change	no change
<i>L. salivarius</i> CECT5713	Sierra 2010 <sup>96</sup>	20	culture	healthy volunteers	none	0	improves	no change
<i>S. boulardii</i> Iyo	Vanhoutte 2006 <sup>97</sup>	30	PCR	healthy volunteers	none	0	no change	no change
<i>Bifido. animalis</i> + <i>Bifido. longum</i>	Zhong 2006, <sup>98</sup> He 2008 <sup>99</sup>	11, 11	FISH, FISH	healthy volunteers	none	7 d, 7d	no change, modifies	no change, no change
<i>L. acidophilic</i> + <i>Bifido. lactis</i>	Yang 2012 <sup>100</sup>	63	culture	healthy but 55% <i>H. pylori</i> +	none	0	restores	alters
<i>L. rhamnosus</i> GG + <i>Bifido. longum</i> Bb536	Mah 2007 <sup>101</sup>	20	FISH	healthy neonates	none	6 mon	no change	no change
<i>L. rhamnosus</i> GG + <i>Bifido. lactis</i> Bb12	Rafter 2007 <sup>102</sup>	38	culture	colon cancer patients or at risk	none	0	no change	no change

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36	<i>L. rhamnosus</i> GG + <i>L. gasseri</i> TMC0356	Kubota 2009 <sup>103</sup>	14	culture FISH TRFLP	healthy, allergy patients	none	0	suppressed changes	alters
	<i>L. paracasei</i> B21060 + <i>L. paracasei</i> B21070 + <i>L. gasseri</i> B21090	Morelli 2003 <sup>104</sup>	12	culture	healthy volunteers	none	3 d	no claims	no change
	<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	Sullivan 2009 <sup>105</sup>	15	culture	chronic fatigue patients	none	4 wk	no change	no change
	<i>L. rhamnosus</i> 271 + <i>L. acidophilus</i> NCFM + <i>L. paracasei</i> 114001 + <i>Bifido. animalis</i> 1017	Engelbrektson 2006 <sup>50</sup>	22	culture TRFLP PCR	healthy volunteers	none	2 wk	no change	no change
	<i>Bifido. animalis</i> lactis + <i>L. delbrueckii</i> I-1632 + <i>L. delbrueckii</i> I-1519 + <i>L. lactis</i> cremoris	McNulty 2011 <sup>106</sup>	7	PCR	healthy twins volunteers	none	4 wk	no change	no change
	<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Vitali 2012 <sup>14</sup>	15	PCR	healthy pregnant volunteers	none	0	modulates	no change

37 \*including strain (when reported)

38 Abbreviations: FISH, fluorescence *in situ* hybridization analysis; TRFLP, terminal restriction fragment  
39 length polymorphism analysis; PCR, polymerase chain reaction  
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**Table 4.** Comparison of the ability of probiotic to restore or improve dysbiosis with ranked clinical efficacy for various disease indications.

Probiotic*	Restored normal microbiota *	Altered normal microbiota*	Ranked net evidence for efficacy**							
			AAD	CDI	IBD	IBS	TD	H pylori	Vaginitis/BV	Acute Ped diar
<b>Restores microbiota</b>										
<i>Clostr. butyricum</i> <i>MIYAIRI</i>	yes	nd	-						-	
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	yes	nd	0	-						
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	yes	nd				-				
<i>Bifido. longum</i>	yes	no			-	+				
<i>L. acidophilus</i> + <i>L. acidophilus</i> + <i>Bifido. bifidum</i> + <i>Bifido. animalis</i>	yes	nd								
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i> (2)	yes	no								
<i>S. boulardii</i> lyo	partial	yes	++	++	++	0	+	-		++
<i>L. rhamnosus</i> GG	partial	nd	-	-	-	0	0	-	0	++
<i>L. acidophilus</i>	partial	no	++			++	-	-	+	0
<i>L. acidophilus</i> + <i>L. bifidus</i> + <i>L. rhamnosus</i>	partial	nd								
<b>Alters microbiota</b>										
<i>E. coli</i> Nissle	nd	yes			-					+
<i>L. casei</i> (DN114001 or Lcr35)	nd	yes	+					0	+	++
<i>L. rhamnosus</i> GR1 + <i>L. fermentum</i> RC14	nd	yes							++	
<i>L. plantarum</i> 8PA3 + <i>Bifido. bifidum</i>	nd	yes								
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii</i> <i>shermanii</i> JS + <i>Bifido. breve</i> Bb99	nd	yes				++				
<i>L. acidophilus</i> + <i>L. plantarum</i> + <i>L. rhamnosus</i> + <i>Bifido</i> <i>bifidum</i>	nd-	yes								

<i>L. brevis</i> CD2 + <i>L. salivarius</i> FV2 + <i>L. plantarum</i> FV9	nd	yes								+	
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp.</i> <i>bulgaricus</i> + <i>L. plantarum</i> , <i>Bifido.</i> <i>longum</i> , <i>Bifido.</i> <i>infantis</i> , <i>Bifido.</i> <i>breve</i>	nd	yes	-		++	+					++
<b>No effect on microbiota</b>											
<i>B. clausii</i>	nd	nd							-		-
<i>L. plantarum</i> 299v	nd	no	-	-		-					
<i>Bifido. lactis</i>	nd	no	+								0
<i>Bifido. breve</i>	no	no									
<i>L. acidophilus</i> + <i>Bifido. longum</i>	no	--									
<i>L. rhamnosus</i> 19070-2 + <i>L. reuteri</i> DSM	nd	no									0
<i>L. casei</i> + <i>Bifido. breve</i>	nd	no									
<i>L. paracasei</i> + <i>L. acidophilus</i> + <i>Bifido animalis</i>	nd	no									
<b>Pharmacokinetic only</b>											
<i>L. reuteri</i> 55730	nd	nd									+
<i>L. johnsonii</i> La1	nd	nd			-				+		
<i>L. salivarius</i> UCC4331	nd	nd				-					
<i>Bifido. infantis</i> 35624	nd	nd				0					
<i>Bifido. bifidum</i> MIMBb75	nd	nd				+					
<i>L. rhamnosus</i> + <i>Bifido. longum</i>	nd	nd									

\*including strain (when reported)

\*\* **Rank:** ++,  $\geq 2$  net RCTs (randomized controlled trials) with significant protective efficacy; +, only one net protective RCT; 0, equal number of significant and non-significant RCTs; -,  $\geq 1$  net non-significant RCT. Blank indicates no RCT done for the disease indication.

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3 **Abbreviations:** nd, not determined; AAD, antibiotic associated diarrhea; CDI, Clostridium  
4 difficile infections; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; TD,  
5 traveler's diarrhea; BV, bacterial vaginosis; Acute Ped Diar, treatment of acute pediatric  
6 diarrhea.  
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6 **Contributorship statement:** The listed author made substantial contributions to the conception  
7 and design, acquisition of data, analysis and interpretation of the data, drafting the article and  
8 revising it critically for important intellectual content and had final approval of the version to be  
9 published. No person or persons other than the author has contributed significantly to its  
10 preparation. This manuscript (in full or in part) is not under consideration for publication in any  
11 other journal, nor is a duplicative paper.  
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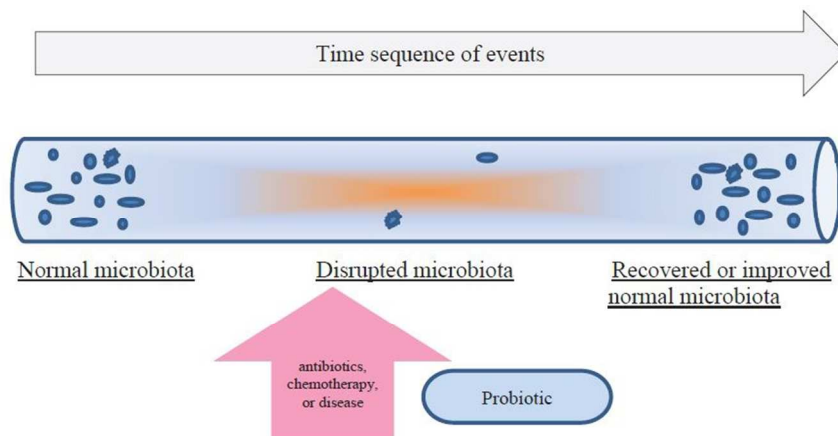
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16 **Figure 1.** Time sequence of events and three models of study designs determining three different  
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18 degrees of dysbiosis correction by probiotics.  
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**Figure 2.** Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota

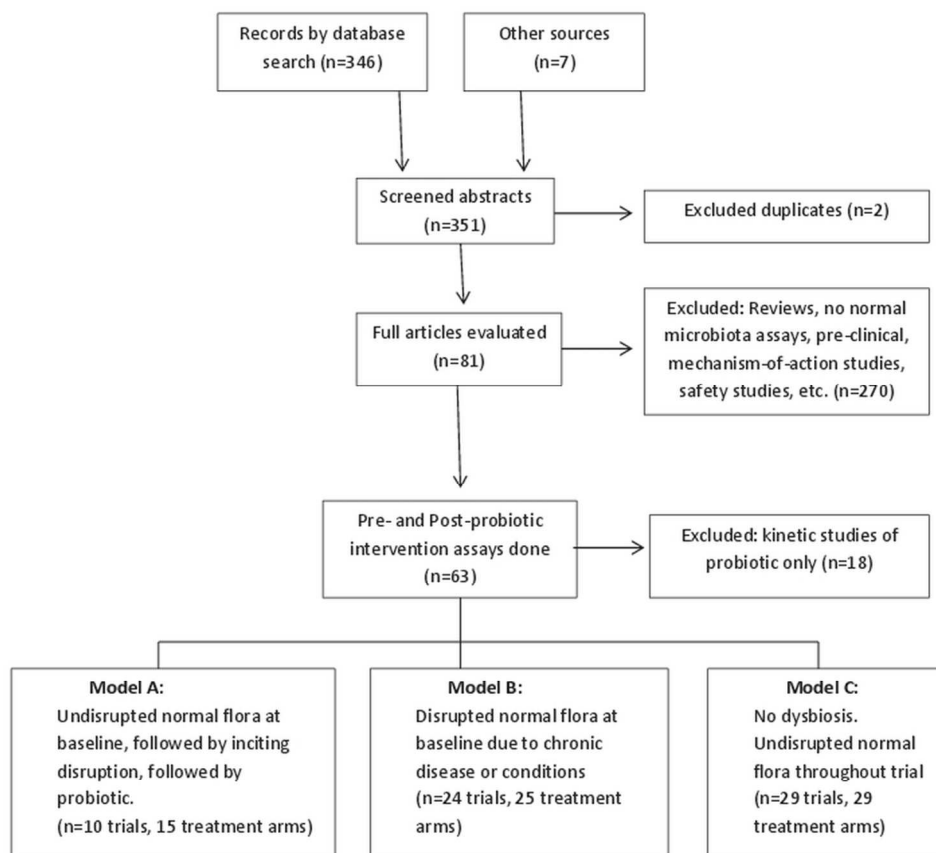
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Model	Type of population enrolled	Dysbiosis at baseline	Time microbiota disrupted	Probiotic or control intervention	Potential outcomes
A	Healthy volunteers or at-risk patients	no	post-baseline	preventive	restoration
B	Patients with active disease at enrollment	yes	pre-baseline	treatment	altered or improved
C	Healthy volunteers	no	not disrupted	preventive	altered

Time sequence of events and three models of study designs determining three different degrees of dysbiosis correction by probiotics.  
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Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota.  
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# PRISMA 2009 Checklist

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Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both. <b>This is a systematic review</b>	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known. <b>Probiotics are promising candidates to prevent or treat disease, but are typically supported by structure/function claims in most countries. The function claim for the restoration of normal microbiota is commonly cited in efficacy trials, but the evidence for this claim has not been examined systematically for all probiotic strains. Differences in study populations and study design effect the type of conclusions that can be drawn. This is the first systematic review and proof of principle of this type of analysis for the function claim of dysbiosis.</b>	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). <b>A comprehensive literature review of the evidence from randomized controlled trials will discuss the strength of the evidence for the restoration or improvement of dysbiosis by specific probiotic strain. The interventions include probiotic or control (typically placebo) given for a specific time enrolled in clinical trials for either the prevention or treatment of disease. All trials which stated some impact on the normal microbiota will be reviewed and analyzed for the ability to document changes in the normal microbiota. The outcomes are the degree of dysbiosis restoration depending upon the study design and type of enrolled participants.</b>	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. <b>Review protocol is described in Methods section of paper. Prospero registration number is: CRD42014007224</b>	5-9
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. <b>Participants: without restriction, any enrolled in clinical trial (adults and pediatrics)</b>	6





# PRISMA 2009 Checklist

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		<p><b>Interventions: all probiotics</b>  <b>Comparisons: controlled (typically placebo)</b>  <b>Outcomes: microbiologic assays of the intestinal flora or microbiota</b>  <b>Study design: required to have pre-intervention (baseline) and post-intervention microbiological assays</b>  <b>Length of follow-up: unrestricted</b>  <b>Language: unrestricted</b>  <b>Publication and years considered: peer-reviewed publications from PubMed (1985-2013, unless otherwise noted), EMBASE, Cochrane Database (1990-2013), CINAHL, AMED, ISI Web of Science (2000-2013). On-line trial registries: (Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.</b></p>	
Information sources	7	<p>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.</p> <p><b>See item above</b></p>	6
Search	8	<p>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</p> <p><b>All probiotics + health claims, structure/function claims, normal microbiota, normal intestinal flora, dysbiosis, pharmacokinetics, metagenomics, dietary supplements, randomized controlled trials, antibiotic-associated diarrhea, Clostridium difficile infections, H. pylori treatments, inflammatory bowel disease, irritable bowel disease, travelers diarrhea, bacterial vaginosis or vaginitis, treatment of pediatric acute diarrhea, healthy volunteer trials and specific probiotic strains. (PubMed)</b></p>	5-6
Study selection	9	<p>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</p> <p><b>Screening:</b>  <b>Eligibility: Must have at least one pre-intervention (baseline) microbiological assay of normal flora or metagenomic analysis and one post-intervention (post-probiotic) assay. Genus and species of probiotic strain(s) provided. Normal microbiota assayed during a randomized, controlled trial.</b>  <b>Excluded: pre-clinical studies, safety studies, reviews, mechanism of action studies, case reports or case series, duplicate reports, unspecified type of probiotics.</b></p>	6-7
Data collection process	10	<p>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</p> <p><b>Pilot data extraction form used modified from standard meta-analysis data extraction form (McFarland and Goh 2013, World J Gastroenterol). Questionable results were queried from original authors of papers.</b></p>	6
Data items	11	<p>List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.</p> <p><b>The timing and type of microbiologic assays of intestinal or vaginal microbiota were collected. As the literature review has a length inclusion period (1985-2013), the types of microbiologic assays have evolved, but all types were included from basic microbiologic assays to metagenomic profiling. The type of probiotic intervention was collected by genus, species and strain (if stated in paper). Types of normal microbiota assays varied by technique. The patient population (healthy volunteers, acute disease or chronic disease) was also collected. Also collected: study size, type of disruptive factor, follow-up duration, stated claims in</b></p>	7-8



## PRISMA 2009 Checklist

		paper.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. <b>Quality of study design (restoration, improvement or no dysbiosis) was used when assessing quality of individual studies. These were then analyzed by stratification on the quality of study design.</b>	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). <b>NA, No pooled RR or DWMs used in this systematic review.</b>	na
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis. <b>NA, No pooled RR or DWMs used in this systematic review.</b>	na

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). <b>All studies with microbiota were included to limit bias, but no measurement for publication bias was done for this systematic review.</b>	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. <b>association of degree of dysbiosis correction with clinical efficacy by probiotic strain(s)</b>	8-9
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. <b>See flow diagram, Figure 2.</b>	25
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. <b>Extracted data were cited in tables. See paper-Tables 1-4.</b>	26-32
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). <b>Presented in Discussion section.</b>	11-15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. <b>Cited in Tables 1-4.</b>	26-32
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. <b>NA, this is a systematic review, not a meta-analysis.</b>	na
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15). <small>For peer review only: <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a></small>	18-19



# PRISMA 2009 Checklist

		<b>Presented in Discussion section (bias due to study design).</b>	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	15-17
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). <b>Strength of the evidence is provided in the Results section.</b> <b>Relevance to key groups is in the Discussion section.</b>	19, 22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). <b>Provided in Discussion section and “Opportunities for Future Research” section and ‘Strengths and Weaknesses” section.</b>	21-22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. <b>The weight of the evidence for the function claim that probiotics can improve or restore normal microbiota is strong for a few probiotic strains, but in general, more confirmatory studies that are properly timed and designed are required for the majority of probiotic strains.</b>	20-21
<b>FUNDING</b>			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. <b>This review was unfunded.</b>	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

# BMJ Open

## Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review.

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005047.R2
Article Type:	Research
Date Submitted by the Author:	29-Jul-2014
Complete List of Authors:	McFarland, Lynne; Puget Sound VA, Health Services RandD
<b>Primary Subject Heading</b>:	Research methods
Secondary Subject Heading:	Evidence based practice, Gastroenterology and hepatology, Infectious diseases, Nutrition and metabolism, Paediatrics
Keywords:	Microbiology < BASIC SCIENCES, CLINICAL PHARMACOLOGY, Adult gastroenterology < GASTROENTEROLOGY, Functional bowel disorders < GASTROENTEROLOGY, Gastrointestinal infections < GASTROENTEROLOGY, Inflammatory bowel disease < GASTROENTEROLOGY

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4 **Use of probiotics to correct dysbiosis of normal microbiota following disease**  
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7 **or disruptive events: a systematic review.**  
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11 Lynne V. McFarland, PhD, MS

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27 **Disclaimer:** *The findings and conclusions in this study are those of the author*  
28 *and do not represent the official position of the University.*  
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40 ever it may be located; and vi) licence any third part to do any or all of the above.  
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44 **Word Count** 6731

45 **Figures:** 2

46 **Tables:** 4  
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## ABSTRACT

**Objective:** To assess the evidence for the claim probiotics can correct dysbiosis of the normal microbiota resulting from disease or disruptive events.

**Setting:** Systematic review of published clinical trials of patients receiving a probiotic intervention for the prevention or treatment of various diseases.

**Data sources:** Sources searched (1985-2013): PubMed, EMBASE, Cochrane Database of Systematic Reviews, CINAHL, AMED, and ISI Web of Science. Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.

**Review methods:** Included studies were randomized clinical trials of probiotic interventions having microbiologic assays. Studies were evaluated following PRISMA guidelines for specific probiotic strains. A standard data extraction form was used to collect the raw data.

**Outcome measures:** The primary outcome is the degree of microbiota correction by specific probiotic strains. Secondary outcome was the association between the degree of dysbiosis correction and clinical efficacy.

**Results:** The review of the literature found three distinct study designs: Model A (restoration) assayed patients enrolled with a healthy, undisturbed microbiota and then assayed post-disruptive event and probiotic therapy; Model B (alteration) assayed patients with pre-existing disrupted microbiota and then post-probiotic therapy; Model C (no dysbiosis) assayed volunteers with no disruptive event pre and post-probiotic. From a total of 63 trials, 83% of the probiotic products using Model A restored the microbiota, 56% using Model B improved the microbiota and only 21% using Model C had any effect on microbiota. Clinical efficacy was more commonly associated with strains capable of restoration of the normal microbiota.

**Conclusions:** The ability to assess the degree of dysbiosis improvement is dependent upon the enrolled population and the timing of microbiologic assays. The functional claim for correcting dysbiosis is poorly supported for most probiotic strains and requires further research.

**Systematic review registration:** PROSPERO (CRD42014007224)

## Strengths and Limitations

### Strengths include:

- A comprehensive review of the published literature from 1985-2013
- Literature search unrestricted by language or country
- Analysis of study designs resulted in novel strategy to limit bias and classify outcomes
- Three types of outcomes of dysbiosis applied to evidence-based studies of specific probiotic strains
- Author has over 40 years of research experience in the probiotic field

### Limitations include:

- Pooled clinical trials using different study populations
- Pooled probiotic doses and regimens
- Indirect evidence linking probiotic strains and dysbiosis
- Review done by sole author

## INTRODUCTION

The popularity of probiotics has expanded exponentially recently, but along with their increased use, debate rages on how probiotics should be regulated and whether probiotics should be considered as a medical food, drug or a food supplement. In the U.S., probiotics are typically available as dietary supplements and thus are limited to 'structure or function' health claims and, unlike prescription drugs, are not permitted to claim to 'treat' or 'cure' disease. In Europe and the United Kingdom, probiotics are allowed to have health or function claims. These claims are required to be supported by well-conducted human trials in the targeted population or in healthy volunteers, but the European Food Safety Authority (EFSA) has rejected >80% of claims submitted to them.<sup>1-3</sup> In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect.<sup>4</sup> One such functional claim made for probiotic products is they correct dysbiosis (or the disruption of bacterial and fungal species after antibiotics or other disruptive exposures) and thus may be beneficial to maintain health. Probiotics are active during this susceptible window from the time of the disruptive event to the time when normal microbiota is restored. A wide variety of mechanisms-of-action have been documented for probiotics (ranging from blocking pathogen attachment sites, destruction of the pathogen by bacteriocins or proteases that degrade toxins, to regulation of the immune system),<sup>5,6</sup> and while clinical evidence supports efficacy of some probiotic strains, the evidence linking these mechanisms-of-action to a specific health or function claims is not as clear.

A classic example of the consequence of dysbiosis is antibiotic-associated diarrhea (AAD).<sup>7,8</sup> While antibiotics may be effective in the elimination of pathogenic organisms, a common, unintended effect is the killing or inhibition of beneficial microbes due to shared susceptibility to the antibiotic. One of the many functions for normal microbiota is the ability to resist infection by pathogenic organisms, termed 'colonization resistance'.<sup>9,10</sup> The loss of a sub-population of the normal microbiota, for example, can lead to the loss of the ability to break down fibers and starches into absorbable short chain fatty acids, resulting in high level of undigested carbohydrates, which can trigger diarrhea.<sup>11</sup> Disruption of the normal microbiota has been shown to lead to higher rates of infections in other body systems other than the intestinal tract including the skin,<sup>12,13</sup> vagina,<sup>14,15</sup> respiratory tract,<sup>16,17</sup> and in the buccal cavity.<sup>18-20</sup>



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6 The major challenge to establishing a cause and effect for the improvement of dysbiosis by  
7 probiotics is a lack of a standard definition of 'normal' microbiota. There is substantial inter-  
8 individual variation of the species of microbes present at different body niches, which also varies  
9 by age, geographic area and health status of the host. In addition, a complete accounting of the  
10 microbiota is currently impossible, as there are no assays to detect all of  $>10^{13}$ - $10^{14}$  organisms in  
11 the intestines and standard microbial culturing methods miss 75-95% of these organisms.<sup>21,22</sup>  
12 The development of metagenomics (cataloguing individual and disease-specific bacterial gene  
13 profiles) and the creation of the international Human Microbiome Project ushered in a new era  
14 for our understanding of the complexity of these interactions within the body.<sup>23,24</sup> This paradigm  
15 shift from culturing to metagenomic analysis has expanded our ability to document shifts in  
16 microbial populations to an unparalleled degree, but the interpretation of these shifts continues to  
17 be under debate.<sup>25-28</sup> With the advent of these newer metagenomic tools, the role of probiotics in  
18 the restoration of normal microbiota is being re-visited.<sup>29</sup>  
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32 In light of new guidance documents and recommendations, the goal of this systematic review is  
33 to determine how claims for the restoration of the normal microbiota and the correction of  
34 dysbiosis have been studied using well-designed trials and which probiotic strains have  
35 evidence-based data to support these claims.  
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## 42 **METHODS**

### 43 **Study Objective**

44 To systematically review the literature to analyse the evidence for the claim probiotics can  
45 correct dysbiosis of the normal microbiota from randomised controlled trials.  
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### 52 **Search Strategy**

53 Search terms included: probiotics + health claims, restoring normal microbiota, dysbiosis,  
54 normal microbiota, pharmacokinetics, metagenomics, probiotics, dietary supplements,  
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3 randomized controlled trials, antibiotic associated diarrhea (AAD), *Clostridium difficile* infection  
4 (CDI), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), traveler's diarrhea  
5 (TD), eradication of *Helicobacter pylori*, bacterial vaginosis or vaginitis, treatment of acute  
6 pediatric diarrhea, and specific probiotic strains or products. Search strategies were broad-based  
7 initially, then narrowed to clinical trials with probiotics.  
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### 13 14 **Data Sources**

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16 PubMed (1985-2013), EMBASE (1985-2013), Cochrane Database of Systematic Reviews  
17 (1990-2013), CINAHL (1985-2013), AMED (1985-2013), and ISI Web of Science (2000-2013).  
18 Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled  
19 trials (<http://www.cochrane.org>), MetaRegister of Controlled Trials (<http://www.controlled->  
20 [trials.com/mrct](http://www.controlled-trials.com/mrct)) and National Institutes of Health (<http://www.clinicaltrials.gov>).  
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### 27 **Criteria for study selection and data extraction**

28 Abstracts of all citations were reviewed by a single author and rated for inclusion for randomized  
29 controlled trials of probiotic treatments. Full articles were retrieved if normal microbiota assays  
30 were mentioned. Non-English language trials were translated and included whenever possible.  
31 Exclusion criteria included pre-clinical studies (animal models or *in vitro* assays), safety or phase  
32 2 studies, reviews, efficacy trials with no assays for normal microbiota species, metagenomic  
33 methods only, mechanism of action of normal microbiota or probiotic, cross-sectional surveys,  
34 case reports or case series, duplicate reports, or trials of unspecified types of probiotics. All  
35 pharmacokinetic studies in humans were reviewed, as abstracts often did not include normal  
36 microbiota assay data. Data extraction and the review process followed the PRISMA statement  
37 guidelines using a 27-item checklist and flow diagram.<sup>30</sup> A standardized data extraction form  
38 was used to collect data on the probiotic (strain type, daily dose, duration), type of controls  
39 (placebo, active or no treatment), study design (status of microbiota at baseline and follow-up  
40 times), type of microbiota assay (microbial culturing, molecular biomarkers, etc.), enrolled study  
41 population (adult vs. pediatric, healthy volunteers, disease condition), type and timing of  
42 disruptive agent (antibiotics, chemotherapy, etc.), study size and attrition, outcome assessment  
43 (efficacy and/or microbiota status at end of study, adverse events) and type of health claim.  
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## Outcomes and definitions

The primary outcome is the degree of microbiota correction or improvement by specific probiotic strain(s). The secondary outcome is the association between the degree of dysbiosis correction and the net efficacy found from randomized controlled trials of probiotic interventions. Dysbiosis is defined as an alteration or disruption of the normal microbiota (bacterial or fungal species) due to exposure of an disruptive factor (such as antibiotics, chronic disease, stress, medical procedures or medications, etc.). As there is no current standard definition of 'normal' microbiota, for this review, restoration of normal microbiota is defined as a return to the assayed microbial species or profile taken from a healthy individual (before a disruptive event has occurred). Included studies are required to have at least a pre-probiotic treatment assay and a post-probiotic treatment assay. A variety of microbial assays were available during the search period (1985-2013), including documentation of the microbiota by either microbial cultures, or metagenomic methods [16s rRNA-targeted probes using fluorescent *in situ* hybridization (FISH) or other polymerase chain reaction (PCR) technique]<sup>8,21,28,31</sup> or by indirect methods (Nugent scores).<sup>15</sup> Nugent scores (ranged 0-10) are used to diagnose bacterial vaginosis (scores  $\geq 7$ ) or normal vaginal microbiota (scores 0-3) based on the quantitated morphotypes of small gram negative rods (*G. vaginalis/Bacteroides* spp.) and curved gram negative rods (*Mobiluncus* spp.) from gram stains of vaginal discharge smears. Microbial assays of only the strain(s) contained in the probiotic product are considered as pharmacokinetic studies and were not included in the normal microbiota profiles.

**Models of dysbiosis.** To determine the impact on normal microbiota, only direct evidence of microbiota change (species, profiles, diversity indices, or diagnostic criteria) were included and indirect effects were excluded (changes in intestinal enzymes, immune system parameters or disease symptoms). The degree to which dysbiosis was improved is categorized into three levels: (1) recovery of the normal microbiota back to baseline levels; (2) alteration or improvement of the normal microbiota; and (3) no change in normal microbiota.

The literature contained three dysbiosis models: Model A (restoration of the normal microbiota), which assayed patients enrolled with a healthy, undisturbed microbiota and then assayed again after a disruptive event (such as antibiotic exposure) and probiotic therapy occurred; Model B (alteration of the microbiota) assayed patients with pre-existing disrupted microbiota (for

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3 example, pre-existing chronic disease or active disease) and then post-probiotic therapy; Model  
4 C (no dysbiosis) assayed volunteers with no disruptive event (before or during the clinical trial)  
5 at both pre-probiotic and post-probiotic times, as shown in Figure 1. 'Recovery' of the normal  
6 microbiota is defined as a restoration of the microbiota back to a normal healthy baseline.  
7 Recovery may be complete recovery (all assayed microbial levels returned to baseline) or  
8 incomplete recovery (partial recovery of some microbial strains, but not all returned to baseline  
9 levels). In studies enrolling subjects with dysbiosis at baseline (typically due to chronic  
10 diseases), it is not possible to show a restoration to normal microbiota levels because a normal,  
11 undisturbed microbiota was not present in these types of study subjects at the time of enrollment.  
12 Therefore, the strongest claim possible for Model B designs is for an 'alteration or improvement'  
13 of the microbiota. Only data from the probiotic-exposed subjects were analysed in this paper.  
14 Data from the control groups were used to confirm dysbiosis for subjects with chronic diseases  
15 or after a disruptive exposure, such as antibiotics or chemotherapy, unaffected by probiotic  
16 exposure.<sup>32-34</sup>  
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### 30 **Assessment of methodological strength and quality**

31 The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system  
32 for rating overall study quality will be used for each probiotic strain or type (single strains and  
33 mixtures of strains).<sup>35</sup> Recommendation for the support of the claim of each probiotic strain or  
34 mixture can be assessed by the overall strength of the evidence ["strong", many randomized  
35 controlled trials show significant recovery of the microbiota, or "moderate" only one randomized  
36 controlled trial; or "weak", only case series or reports, limited number of small trials, *etc.*].  
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44 Quality of the evidence is based on study design and graded as "high quality" (well-defined  
45 study design for determining restoration with normal microbiota, Model A), or "moderate  
46 quality" (disrupted microbiota at baseline, Model B), or "low quality" (no disruptive event  
47 occurred, Model C). Measurement of publication bias was not assessed for this review, as  
48 pooled outcome estimates of efficacy were not done, as typical in meta-analysis, but all studies  
49 with assays of microbiota were included to limit bias.  
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### 56 **Net efficacy rating**

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3 To determine if the ability to correct dysbiosis is associated with clinical efficacy, the published  
4 literature for randomized controlled trials (RCTs) or meta-analyses of probiotics for various  
5 disease indications, including antibiotic associated diarrhea (AAD),<sup>5,36,37</sup> *Clostridium difficile*  
6 infection (CDI),<sup>5,38</sup> inflammatory bowel disease (IBD),<sup>39</sup> irritable bowel syndrome (IBS),<sup>40</sup>  
7 traveler's diarrhea (TD),<sup>41</sup> eradication of *Helicobacter pylori* (Hp),<sup>36,37</sup> bacterial vaginosis (BV)  
8 or vaginitis,<sup>42</sup> and treatment of acute pediatric diarrhea was reviewed.<sup>43-45</sup> The net rank was  
9 calculated by subtracting the number of RCTs showing non-significant or equivalent efficacy  
10 from the number of RCTs having significant efficacies. The ranks were categorized as follows:  
11 ++,  $\geq 2$  net RCTs showing significant efficacy; +, net of one RCT showing significant efficacy; 0,  
12 equal number of RCTs showing significant and non-significant efficacy results and -,  $\geq 1$  net  
13 negative or non-significant RCTs. Probiotics with no RCTs were not ranked.  
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## 25 RESULTS

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27 A review of the literature from 1985-2013 found 353 articles that dealt with probiotic treatments  
28 and their potential effect on normal microbiota.  
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### 33 Excluded studies

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35 As shown in Figure 2, a total of 272 articles were excluded for the following reasons: reviews  
36 (n=116), probiotic efficacy studies with no data on normal microbiota assays (n=54), animal  
37 models of probiotics and changes in microbiota (n=38), metagenomic or microbiota methods  
38 only (n=17), studies on normal microbiota but with no use of probiotics (n=14), *in vitro* assays of  
39 microbiota (n=10), duplicative reports (n=2) or miscellaneous (n=21), which included probiotic  
40 mechanism of action studies, safety studies, duplicative reports, cross-sectional surveys and two  
41 with poorly described probiotic interventions.<sup>46,47</sup> A total of 81 full articles were reviewed which  
42 mentioned changes in normal microbiota or indicated a health claim for probiotics and effects on  
43 normal microbiota.  
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52 Probiotic pharmacokinetic studies (n=18) reporting concentrations of probiotic strains before and  
53 post-treatment, but did not assaying for other species of normal microbiota were excluded.  
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55 While several studies using this study design claim probiotics had an impact on normal  
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3 microbiota, type of data generated is pharmacokinetic behavior of the probiotics themselves and  
4 not the normal microbiota. Several studies stated that the normal microbiota was altered because  
5 an increase in various bacterial species was observed after the probiotics were given, but the  
6 species assayed were those contained in the probiotic product, so an increase is not unexpected.  
7 Pharmacokinetic studies have documented that probiotic strains taken orally can survive transit  
8 through the intestinal tract with recovery rates in feces ranging from <1% to 22%.<sup>48,49</sup> These  
9 pharmacokinetic studies were excluded from this analysis, as they did not assay other types of  
10 normal microbiota not found in the probiotic product.  
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### 20 **Included studies**

21 Of the 63 included clinical trials, five trials had multiple treatment arms, which resulted in a total  
22 of 68 treatment arms for analysis. Engelbrekton et al. tested a mixture of 5 probiotic strains in  
23 volunteers exposed to antibiotics and also tested a mixture of 4 probiotic strains in healthy  
24 volunteers with no antibiotic exposure.<sup>50</sup> Zoppi et al. had eight different treatment arms in his  
25 study, and probiotic arms were included in our analysis [*Saccharomyces boulardii* (*S. boulardii*)  
26 alone and *Lactobacillus* (*L.*) *rhamnosus* GG alone], a mixture of two probiotics (*L. acidophilus*  
27 and *Bifido. bifidum*) and a mixture of three probiotic strains (*L. acidophilus*, *L. rhamnosus* and  
28 *Bifido. bifidum*).<sup>51</sup> Orrhage et al. had two treatment arms (*Bifido. longum* alone and a mixture of  
29 *Bifido. longum* and *L. acidophilus*).<sup>52</sup> Larsen et al. tested two single probiotics (*Bifido. lactis* and  
30 *L. acidophilus*) in separate treatment arms.<sup>53</sup> Lidbeck et al. gave either enoxacin or clindamycin  
31 and randomized patients to either *L. acidophilus* or placebo.<sup>54</sup>  
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44 **Normal microbiota assay methods.** Of the 69 treatment arms that did normal microbiota assays,  
45 diverse methods were used to profile the microbiota. Many studies used only standard  
46 microbiological culture assays (37, 54%), while others (28, 40%) used techniques to detect non-  
47 cultivatable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16s rRNA  
48 sequencing) or other PCR techniques. Some studies (4, 6%) used an indirect measure of normal  
49 microbiota, using the Nugent score to diagnose bacterial vaginosis, which relies upon gram stain  
50 of the vaginal secretions, vaginal pH and symptoms to characterize if normal microbiota is  
51 present or absent.<sup>15</sup>  
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6 **Probiotic strains.** In the 69 treatment arms, most (36, 52%) used a single strain of probiotic,  
7 while 14 (20%) tested a mix of two probiotic strains and 19 (28%) tested a mix of three or more  
8 probiotic strains. The distribution of single versus multiple strain probiotics did not significant  
9 vary by the model of study design ( $\chi^2_2=2.3$ ,  $P=0.32$ ). Of the 15 restorative (Model A) study  
10 arms, 47% used a single strain of probiotic and 53% used multiple strains. Of the 25 treatment  
11 arms with disrupted microbiota at baseline (Model B), 44% used a single strain and 56% used  
12 multiple strains. Of the 29 study arms with undisturbed microbiota (Model C), 62% used a  
13 single strain and 38% used multiple strains.  
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### 23 **Normal microbiota restoration model (Model A)**

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25 Only 10 studies (with 15 treatment arms) using Model A to determine restoration of the  
26 microbiota were found (Table 1).<sup>32,34,50-52,54-58</sup> The type of enrolled subjects varied from healthy  
27 volunteers to children with untreated respiratory infections, to pediatric cancer patients. For  
28 subjects with acute infections or cancer, baseline assays were done prior to the disrupting agent  
29 (antibiotics or chemotherapy). The number of subjects given probiotics averaged 20/study and  
30 ranged from 5 to 83. In 93%, the disruptive factor was antibiotic exposure and in one study,  
31 chemotherapy caused the microbiota disruption. Only 8 (53%) of the study arms did an assay  
32 during a 1-8 week follow-up period after the probiotic was discontinued.  
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43 Analysis of the probiotic strain(s) separately found only two probiotic products with more than  
44 one randomized controlled trial. The probiotic mix of *L. acidophilus* and *Bifido. bifidum* showed  
45 a complete restoration in one study, but only a partial recovery in the other. (Strength: strong,  
46 Quality: high). The probiotic mix of *L. acidophilus* (2 strains) with *Bifido. bifidum* and *Bifido.*  
47 *animalis* showed complete restoration in one study, but only a partial recovery in the other.  
48 (Strength: strong, Quality: high). Five other probiotic products with only one supporting clinical  
49 trial showed microbiota restoration (*Bifido. longum*, *Clostridium butyricum*, *L. acidophilus*, mix  
50 of *L. acidophilus* with *L. paracasei* and *Bifido. lactis*, and the mix of *L. acidophilus* with *L.*  
51 *paracasei* and *Bifido. bifidum* and two strains of *Bifido. lactis*). (Strength: moderate, Quality:  
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3 high). Three probiotic products with one supporting clinical trial showed partial restoration (*S.*  
4 *boulevardii*, *L. rhamnosus* GG, mix of *L. rhamnosus* with *L. bifidus* and *L. acidophilus*), (Strength:  
5 moderate, Quality: high). Only two probiotic products using Model A showed no change in the  
6 microbiota (*Bifido. breve* and a mix of *L. acidophilus* and *Bifido. longum*). (Strength: moderate,  
7 Quality: high). In summary, 10 of 12 (83%) of the probiotic products showed complete or partial  
8 restoration of the normal microbiota.  
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17 Of the 11 probiotic products with claims of 'restores or improves normal microbiota', 10 (91%)  
18 were supported by this review, but only seven showed complete restoration and five had partial  
19 restoration of the microbiota (Table 1). The mixture of *L. acidophilus* and *Bifido. longum* did  
20 not show any changes in the microbiota. Wada et al. claimed *Bifido. breve* 'enhanced intestinal  
21 anaerobes', but this was only compared to the placebo group.<sup>32</sup> Their data showed chemotherapy  
22 is a disruptive event, resulting in more Enterobacteria in the intestine in the placebo group, but  
23 there were no significant differences seen by the end of the 8 week follow-up in either the  
24 probiotic or the placebo group compared to baseline microbiota levels.  
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### 35 **Disrupted normal microbiota at baseline studies (Model B)**

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37 Twenty-four studies (with 25 treatment arms) used Model B that enrolled subjects with a pre-  
38 existing disrupted microbiota related to ongoing disease or conditions (Table 2).<sup>33,53,59-80</sup> The  
39 number of subjects given probiotics averaged  $23 \pm 16$ /study and ranged from 7-83 participants.  
40 The types of pre-existing factors that disrupted the microbiota included atopic dermatitis  
41 patients, allergies, cirrhosis, bacterial vaginosis, irritable bowel syndrome, inflammatory bowel  
42 disease (ulcerative colitis and pouchitis), idiopathic diarrhea, enteral feeding, short-bowel  
43 syndrome and colon cancer. Only 10 (40%) of the study arms did an assay during the post-  
44 probiotic follow-up period.  
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55 Three of the probiotics had multiple clinical trials to support the claim of an improvement in the  
56 microbiota due to the probiotic. *S. boulevardii* was used in two trials either with enteral fed  
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3 patients or patients with active diarrhea and found an improvement in the habitual microbiota in  
4 the patients with active diarrhea<sup>66</sup>, but only showed indirect evidence of short-chain fatty acid  
5 changes in the other study.<sup>65</sup> (Strength: strong, Quality: moderate) A mix of four probiotic  
6 strains (2 strains of *L. rhamnosus*, *P. freudenreichii* + *Bifido. breve*) showed improved  
7 microbiota in two clinical trials.<sup>74,75</sup> (Strength: strong, Quality: moderate) Of four clinical trials  
8 testing a mixture of seven probiotic strains, two showed no significant change in microbiota<sup>77,78</sup>,  
9 one showed more anaerobes post-probiotic treatment<sup>79</sup> and one found a reduction in *Bacteroides*  
10 species.<sup>80</sup> (Strength: strong, Quality: moderate) Three clinical trials determined there were no  
11 significant changes due to *L. plantarum* 299v.<sup>62-64</sup> (Strength: strong, Quality: moderate). Of those  
12 probiotics with only one supporting clinical trial (Strength: moderate, Quality: moderate), two  
13 single probiotic strains (*E. coli* Nissle and *L. casei rhamnosus*) and five different mixtures of  
14 probiotic strains support the claim that the probiotic alters the microbiota (Table 2). In summary,  
15 10 of 18 (56%) probiotic products altered or improved microbiota in individuals with pre-  
16 existing disease.  
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32 Of the 25 treatment arms, the paper's claim was confirmed in 14 (56%) of the studies. There was  
33 no significant change in the microbiota due to the probiotic in nine treatment arms and only an  
34 alteration of the microbiota in five others (Table 2). Our review disagreed with the claimed  
35 outcomes in 11 (46%) of the other treatment arms. In seven treatment arms, it was claimed the  
36 tested probiotic 'restored normal microbiota', but it is uncertain how this conclusion was reached,  
37 since there was no time when a normal undisrupted microbiota was present. Of the seven studies  
38 that claimed their probiotic 'restored' normal microbiota, our analysis determined none were  
39 capable of documenting restoration, but it is confirmed probiotics improved or altered the  
40 microbiota in these studies. Four studies claimed the probiotic 'altered or improved' normal  
41 microbiota, but this review found no significant differences when post-probiotic and baseline  
42 assays were compared for the probiotic groups. Girard-Pipau et al. concluded that *S. boulardii*  
43 'altered normal flora' because more gram positive anaerobes were seen in the probiotic group  
44 compared to the controls and an increase in three short-chain fatty acids were observed in the *S.*  
45 *boulardii* group.<sup>65</sup> However, when the analysis is restricted to trends observed in the probiotic  
46 group only, no significant differences were observed in pre-probiotic versus post-probiotic  
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3 microbiota profiles. Venturi et al. concluded that the mix of seven probiotic strains enhanced the  
4 concentration of some beneficial strains in the intestines.<sup>77</sup> However, the only strains having a  
5 significant increase were those contained in the probiotic mix, and not specifically normal  
6 microbiota of the host. As this study did not have an undisturbed microbiota baseline, the  
7 increased numbers of Lactobacilli and Bifidobacteria may not have reflected their normal levels.  
8 Van der Aa et al. claimed that *Bifido. breve* 'successfully modulates the intestinal flora', but no  
9 significant changes were observed in the probiotic group when comparing the baseline to the  
10 post-probiotic levels.<sup>59</sup> Odamaki et al. did show an increase in Faecalibacterium ssp. and  
11 *Bacteroides fragilis* ssp. at the end of *Bifido. longum* BB536 treatment, but the same increase  
12 was also observed in the placebo group.<sup>33</sup>  
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### 24 **Undisrupted normal microbiota studies (Model C)**

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27 Twenty nine trials enrolled healthy adults who had no disruptive factor present during the study  
28 (either no antibiotic or no medication exposure or presence of acute or chronic disease) that  
29 might impact normal microbiota, as shown in Table 3.<sup>14,49,50,81-106</sup> The average number of  
30 subjects given probiotics was 23/study and ranged from 7 to 160/study. Of the 29 study arms,  
31 assays were taken during a follow-up period in only 52%. Fujiwara et al. cultured seven healthy  
32 volunteers and found Enterobacteriaceae and Clostridial species post-*Bifido. longum* was  
33 reduced by 10<sup>1</sup>/g compared to baseline (P<0.03), but no other changes in the microbiota were  
34 detected.<sup>84</sup> Karlsson et al. found a significant increase in intestinal diversity in nine male  
35 volunteers with atherosclerosis given *L. plantarum* 299v, but because terminal restriction  
36 fragment length polymorphism assays were used instead of cultures for bacterial species, the  
37 specific changes in the microbiota species could not be determined.<sup>94</sup> Yang and Sheu cultured 63  
38 children (55% with *H. pylori*) given a yogurt with *L. acidophilus* and *Bifido. lactis* but only  
39 found a decrease in *E. coli* counts in the *H. pylori* negative children sub-group, no significant  
40 changes in normal microbiota was found in the *H. pylori* positive children.<sup>100</sup> Kubota et al.  
41 assayed 29 subjects with Japanese cedar pollen allergy and found milk fermented with *L.*  
42 *rhamnosus* GG and *L. gasseri* TMC0356 suppressed microbiota changes (less intestinal profile  
43 changes), but could not determine specific bacterial species changes due to the type of assay used  
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3 (FISH and TRFLP).<sup>103</sup> In summary, only 4 of 19 (21%) probiotic products altered microbiota in  
4 healthy individuals who had no disruptive event.  
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10 Of the seven studies that claimed their probiotic(s) 'restored or altered' the normal microbiota,  
11 only four claims were confirmed. Sierra et al. claimed *L. salivarius* given to 20 healthy adults  
12 'improved gut microbiota', but only increased levels of Lactobacilli were found and no other  
13 changes in normal microbiota species were detected. The only other evidence was indirect from  
14 changes observed in immune parameters.<sup>96</sup> He et al. claimed a mixture of *Bifido. longum* and  
15 *Bifido. animalis* 'modified' microbiota, but changes were seen only during the yogurt  
16 administration and not after the one week follow-up period.<sup>99</sup> Vitali et al. claimed that the  
17 mixture of four Lactobacilli strains and three Bifidobacteria strains 'modulated vaginal  
18 microbiota', but the only significant changes were due to an increase in the bacterial species  
19 contained in the probiotic mixture.<sup>14</sup>  
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31 Of the probiotics supported by multiple clinical trials (*Bifido. animalis*, *Bifido. longum*, *L. casei*,  
32 *L. plantarum* 299v, the mixture of *Bifido. animalis* and *Bifido. lactis*), 13 of the trials (87%)  
33 support there is no significant change in normal microbiota if the microbiota is not disrupted.  
34 [Strength: strong, Quality: low]  
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#### 42 **Association of clinical efficacy and normal microbiota restoration**

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44 Few studies concurrently compared clinical efficacy and the ability to restore or improve normal  
45 microbiota after dysbiosis. A synthesis of the literature of RCT for eight common disease  
46 indications was performed and the overall net strength was ranked. Probiotics with the ability to  
47 restore normal microbiota were frequently supported by RCTs for efficacy, as shown in Table 4.  
48 Of the 10 probiotics with evidence for restoration, 7 (70%) also had at least one RCT testing for  
49 at least one of the eight diseases, while 30% did not have any supportive RCTs for efficacy. Of  
50 the 7 probiotics with associated RCTs, only two probiotics (*S. boulardii* and *L. acidophilus*) have  
51 strong evidence for efficacy across most of the disease indications, while five probiotics with the  
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4 ability to restore the microbiota had weak or no evidence of efficacy. For example, *S. boulardii*,  
5 which has studies supporting restoration, has strong evidence for clinical efficacy for AAD  
6 (ranked ++: 11 RCTs had significant results and 6 had non-significant results), CDI (ranked ++:  
7 had two RCTs with significant results), IBD (ranked ++: had two RCTs with significant results),  
8 IBS (ranked 0: had one RCT with significant efficacy and one RCT with non-significant results),  
9 TD (ranked +: 3 RCTs with significant efficacy and 2 with non-significant efficacy), *H. pylori*  
10 eradication (ranked -: 2 RCTs with significant results and 4 with non-significant results) and no  
11 studies for BV. *L. acidophilus*, which partially restored the microbiota in a study, is associated  
12 with clinical efficacy for AAD, IBS and BV, but not for TD or eradication of *H. pylori* and  
13 treatment of acute pediatric diarrhea (ranked ++: had 19 RCTs with significant protection and  
14 five with non-significant results). In contrast, *L. rhamnosus GG*, another probiotic capable of  
15 restoring microbiota, is often cited in meta-analysis as having significant efficacy for AAD. Our  
16 results of an updated review of the literature indicate a net weak evidence rating for clinical  
17 efficacy across all disease indications: AAD (ranked -: 3 RCTs had significant results and 6 had  
18 non-significant results), CDI (ranked -: two RCTs with non-significant results), IBD (ranked -:  
19 one RCT with non-significant results), IBS (ranked 0: 2 RCTs with significant efficacy and two  
20 RCTs with non-significant results), TD (ranked 0: one RCT with significant efficacy and one  
21 with non-significant efficacy), *H. pylori* eradication (ranked -: 3 RCTs with non-significant  
22 results), no RCTs for BV and treatment of acute pediatric diarrhea (ranked ++: 10 RCTs with  
23 significant efficacy and one with non-significant findings).  
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42 Efficacy trials were not done as frequently for probiotics shown to only have the ability to alter  
43 or improve, but not restore, the microbiota after dysbiosis. Of nine probiotics that can alter the  
44 microbiota, 6 (67%) have supporting RCTs for at least one disease, but the diversity of  
45 investigated diseases was more limited. *L. casei* had moderate net strength for AAD and  
46 bacterial vaginosis, but was neutral for the ability to eradicate *H. pylori* and other disease  
47 indications were not tested in RCTs with *L. casei*. The probiotic mixture of *L. reuteri* and *L.*  
48 *fermentum* has strong evidence for bacterial vaginosis, but not for any other disease indications  
49 listed in Table 4.  
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3 Of the eight probiotics not capable of altering or restoring normal microbiota, only *L. plantarum*  
4 299v had RCTs for AAD and IBS, both with net negative or weak strength of clinical efficacy.  
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6 *Bifido. lactis* and the mixture of *L. rhamnosus* and *L. reuteri* had net neutral rankings for efficacy  
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8 for the treatment of acute pediatric diarrhea. The other four probiotic products with no effect on  
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10 normal microbiota lacked any RCTs for clinical efficacy. Studies with *B. clausii* did not assay  
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12 for normal microbiota and had non-significant trial results for *H. pylori* eradication and the  
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14 treatment of pediatric diarrhea.

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17 Of the six probiotics with only pharmacokinetic data on the probiotic itself and no other  
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19 investigation of other normal microbiota strains, five had RCTs showing varying net efficacies  
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21 for different disease indications, as shown in Table 4.

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24 Six popular probiotics (*Bacillus clausii*, *Bifido. infantis*, *L. reuteri*, *L. acidophilus* + *L. helveticus*,  
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26 *L. acidophilus* + *L. casei* and *L. acidophilus* + *Bifido. animalis*) have only clinical efficacy  
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28 RCTs, but have not published studies investigating their role in restoring or improving the  
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30 normal microbiota.

### 31 32 33 34 **Discussion**

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36 Developing and evaluating health or function claims for probiotics is an important issue and is  
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38 now identified as a priority for research by several international organizations, including the  
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40 World Gastroenterology Organization<sup>107</sup> and the American Society for Nutrition.<sup>2</sup> The U.S. Food  
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42 and Drug Administration has struggled with appropriate evidence-based health claims for  
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44 probiotic products and currently recommends the use of structure/function claims, such as  
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46 "maintains bowel regularity", but the claim for restoring normal microbiota is still under  
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48 debate.<sup>108</sup> The European Food Safety Authority (EFSA) provides guidance materials that  
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50 recommend health or function claims for probiotics should have beneficial physiological effects  
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52 and have appropriate scientific trials to substantiate the health claims.<sup>3</sup> Acceptable claims for  
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54 intestinal health may include functional claims (improved transit time, softer stool consistency,  
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56 reduction in gastrointestinal discomfort, defense against pathogens). As it is currently not  
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58 possible to define a standard normal microbiota profile, the EFSA recommends functional claims  
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3 for the restoration of normal microbiota should document a recovery of healthy microbiota and  
4 be accompanied by a beneficial physiological or clinical outcome.<sup>3</sup> In addition, because the  
5 efficacy and mechanisms are strain-specific and may vary by probiotic strain, the evidence must  
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7 be analyzed for each probiotic product individually.<sup>5,6,9,109, 110-112</sup>  
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12 An underappreciated finding was the influence that study design and study populations have on  
13 the interpretation of study outcomes. In the literature, five different types of study designs are  
14 commonly found relating to probiotics. The most common study type is a randomized controlled  
15 trial testing the efficacy and safety outcomes in patients, but these trials did not typically  
16 document the impact of the probiotic on the normal microbiota. The second most common type  
17 of study design is pharmacokinetic studies (documenting recovery of oral dose of probiotic or  
18 increase in probiotic strains post-treatment compared to pre-treatment or clearance of the  
19 probiotic). Even though these kinetic studies did not assay for non-probiotic strains, some  
20 extrapolated their results and concluded some effect or improvement of the normal microbiota  
21 was observed by their probiotic.<sup>19,111</sup> These two first types of study designs do not support  
22 evidence-based conclusions for the restoration or alteration of the normal microbiota and were  
23 excluded from this review.  
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34 Three types of study designs are appropriate for the study of dysbiosis. The first type of study  
35 design had normal microbiota assayed at least twice (at baseline, which was before exposure to a  
36 disruptive event or probiotics and then again during or post-probiotic treatment) to show actual  
37 recovery of assayed normal microbiota back to healthy baseline levels. The second type of study  
38 design started with inappropriate baselines (baseline samples taken after normal microbiota had  
39 been disrupted by chronic disease). For patients with established chronic diseases, there is no  
40 “normal microbiota” baseline in either the probiotic or the control group. Even if baselines are  
41 taken during remission, the microbiota may still be impacted by chronic disease or acute  
42 diarrhea. Studies of probiotics in chronic diseases or acute disease typically report on ‘pre-  
43 probiotic treatment’ and ‘post-probiotic treatment’ and may show significant shifts in microbial  
44 species, but it is uncertain if this reflects a true re-establishment of normal microbiota profiles.  
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46 The third type of study design enrolled healthy volunteers, who were not challenged with  
47 antibiotics (so no normal microbiota disruption occurred), and show only the effect of probiotics  
48 on a healthy microbiota (typically mild or no effects). Control groups were not required for our  
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3 assessment of the impact of probiotics on microbiota, but control groups can document the  
4 degree normal microbiota is disrupted by inciting agents (antibiotic, disease onset, etc.).  
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10 Five single strain probiotics (*Bifido. longum*, *Clost. butyricum*, *L. acidophilus*, *L. rhamnosus* and  
11 *S. boulardii*) and five probiotic mixtures [(*L. acidophilus* + *Bifido. bifidum*), (*L. rhamnosus* + *L.*  
12 *bifidus* + *L. acidophilus*), (*L. acidophilus* + *L. paracasei* + *Bifido. lactis*), (*L. acidophilus*, 2  
13 strains, *Bifido. bifidum*, *Bifido. animalis*) and (*L. acidophilus* + *L. paracasei* + *Bifido. bifidum*  
14 + 2 strains of *Bifido. lactis*)] documented either complete or partial recovery of normal  
15 microbiota (Model A). Only two probiotic mixtures [(2 strain mixture: *L. acidophilus* + *Bifido.*  
16 *bifidum*) and (4 strain mixture: *L. acidophilus*, 2 strains, *Bifido. bifidum*, *Bifido. animalis*)] were  
17 supported by a confirmatory study. Evidence that probiotics may alter or improve normal  
18 microbiota (Model B) was found for three single strain probiotics (*E. coli* Nissle, *S. boulardii*  
19 and *L. casei rhamnosus*) and seven mixtures of 2-7 probiotic strains. Of these ten probiotics  
20 finding alteration of the microbiota, only three had multiple trials [*S. boulardii*, and a four strain  
21 mixture (2 strains of *L. rhamnosus* + *P. freudenreichii* + *Bifido. breve*), and a seven strain  
22 mixture (4 Lactobacilli and 3 Bifidobacteria strains)], but only one had consistent results  
23 showing improvements in the microbiota.<sup>74,75</sup> Clearly, more than one study is needed to confirm  
24 the impact of a probiotic on the normal microbiota. Of the 19 probiotic strains (or mixtures)  
25 studied in healthy volunteers who were not exposed to disruptive factors (Model C), no change  
26 in the normal microbiota was observed for 79%, indicating the robustness of the microbiota.  
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44 Improvement in the normal microbiota by specific probiotic strains seemed to be associated with  
45 better clinical endpoints. Within eight common diseases typically treated with probiotics, more  
46 trials with significant efficacy were associated with probiotic strains shown to restore the normal  
47 microbiota, and only one trial with significant efficacy was found for probiotics that did not alter  
48 the microbiota. However, few probiotics had efficacy trials for all eight diseases and many did  
49 not have any efficacy trials.  
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Some probiotics which have published efficacy trials for various diseases did not have studies investigating the effect of the probiotic on normal microbiota: *Bacillus clausii*, *Bifido. infantis*, *L. brevis*, *L. reuteri*, mix of 2 strains (*L. acidophilus* + *L. helveticus*), mix of 2 strains (*L. acidophilus* + *L. casei*) or (*L. acidophilus* + *Bifido. animalis*), mix of 4 strains [*L. rhamnosus* (2 strains), *Propionibacterium freudenreichii* + *Bifido. animalis*] and mix of 7 strains (*L. sporogens*, *L. bifidum*, *L. bulgaricus*, *L. thermophilus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*).

### Comparison of results with other studies

Other reviews in the literature of claims for probiotics relating to changes in the normal microbiota have focused on the broad issues of regulatory standardization of health or function claims, the use of proper study designs and the challenge of defining biomarkers for a 'healthy microbiota'.<sup>3,29,112</sup> Donovan et al. recommends that health claims for probiotics be supported by well-conducted human trials in the targeted population.<sup>2</sup> These reviews also recommend that gut biomarkers need to be correlated with clinical endpoints, however none of these reviews attempted to do so.<sup>29,112</sup> No prior review has attempted to analyze the association between probiotic strains and their impact on normal microbiota by stratifying on the quality of study design.<sup>111</sup> This review addressed these concerns by analyzing probiotic strains by the quality of the study design and only including trials that assessed the normal microbiota (either by microbial culturing or molecular strain biomarkers) and assessed the degree of dysbiosis improvement with clinical outcomes for each probiotic strain.

### Opportunities for future research

Most of the studies (80%) using Model A to document restoration of the normal microbiota only used microbiologic culturing techniques, which can only detect those organisms that grow in culture. Use of the more advanced molecular metagenomic techniques have found that culturing alone misses up to 95% of these organisms.<sup>21,22</sup> The use of the metagenomic techniques was more common in the studies using Model B (48%) and Model C (45%) study designs, which only addresses potential alteration of the microbiota. Characterization of the microbiota is a



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3 complex issue and a comprehensive accounting of all the bacterial and fungal strains in the body  
4 is beyond our current capabilities. Therefore, any studies of changes to the microbiota are  
5 incomplete at best, but general trends in bacterial phylotypes can be documented using DNA  
6 probes and metagenomic techniques. Differential detection bias may be present due to the  
7 variety of assays used in these studies and should be accounted for in future studies.  
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16 Another suggestion for future studies is to include an appropriate follow-up time period post-  
17 probiotic administration. Fewer than half of the reviewed trials did assays for normal microbiota  
18 during an appropriate follow-up period. As it has been shown that recovery from a disrupting  
19 factor can be prolonged (typically eight weeks),<sup>7,8</sup> and studies that failed to find microbiota  
20 recovery might have detected a return to normal baseline levels if a sufficiently long time was  
21 given for the recovery to have occurred. Future studies should strive to allow time for the  
22 restoration of the normal microbiota to occur.  
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31 As the effects of probiotics are strain specific, and many studies typically only report the genus  
32 and species of the tested probiotic, future reports should include a complete description of the  
33 probiotic to the strain level.<sup>5,112</sup>  
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### 40 **Strengths and weaknesses**

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42 The strengths of this review included the completeness of the search strategy, which reviewed  
43 multiple citation databases, trial registries and author searches, use of established PRISMA  
44 protocols for reviews and the use of an outcome classification scheme for different degrees of  
45 assessment for microbial recovery. This analysis controlled the confounding effects of different  
46 study populations and study designs present in the literature. Pharmacokinetic studies of just the  
47 probiotic strain(s) itself were excluded and only trials that assayed other species found in the  
48 microbiota were included. By applying a standard definition for 'restoring' versus 'improving'  
49 normal microbiota, it is possible to distinguish significant differences by the type of study  
50 designs used and differential effects of the different probiotic strains. Limitations of this review  
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3 include: a single author reviewed and extracted the literature, pooling trials from different  
4 populations (adult versus pediatric) and different probiotic doses and regimens used. Incomplete  
5 retrieval of all studies assessing the effect that probiotics have on human microbiota is also a  
6 potential limitation of any literature search. Another limitation is that dysbiosis improvement and  
7 clinical efficacy for probiotic strains is also indirectly associated, no direct cause and effect  
8 relationship was possible with the types of studies done. Another limitation is the current lack of  
9 a standard definition of what comprises a 'normal microbiota'. The constituents of the  
10 microbiota vary by individual, by age, geographic location and health status of the host. Current  
11 microbiologic techniques are improving, but can not detect all species present in the host.  
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## 22 **Conclusion**

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24 The challenges in recommending a specific probiotic to patients who need to restore or improve  
25 their normal microbiota after a disrupting event occurs is two-fold: one is the diversity of  
26 probiotic products available and second is the varying strength of evidence provided by clinical  
27 trials using different outcome measures and study designs. By grouping studies into three groups  
28 that result in three different degrees of probiotic effect (restoration, improvement or no change),  
29 an overview of the body of evidence is possible. By comparing the strength of the clinical  
30 evidence for common diseases by the degree to which the probiotics could impact the restoration  
31 of the normal microbiota, it became obvious that those probiotics with a greater ability to restore  
32 the microbiota are associated with the strongest strength of clinical efficacy. While this evidence  
33 only indirectly links clinical efficacy with the ability to restore the microbiota, the overall review  
34 of the evidence shows this is an important mechanism of action for probiotics. What becomes  
35 obvious is that more studies are required to conclude which probiotic strains have a beneficial  
36 impact on the normal microbiota, as most strains have only a single clinical trial and many  
37 probiotic products overstate the strength of their claim to restore normal microbiota. These types  
38 of issues should be considered for health care policy makers and researchers for future studies  
39 and for creating guidelines for health/function claims.  
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**Table 1. Model A:** Evidence-based data for restoration of normal microbiota (NM) for 12 probiotics from 10 studies (15 treatment arms).

Probiotic*	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment (wks)	Claims stated in papers	Evidence-based claim
<i>Bifido. breve</i>	Wada 2010 <sup>32</sup>	19	FISH	pediatric cancer patients	chemotherapy	8	enhances anaerobes	no change
<i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	restores	restores
<i>Clost. butyricum</i> MIYAIRI	Seki 2003 <sup>34</sup>	83	culture	pediatric respiratory or GI infections	antibiotics	0	restores	restores
<i>L. acidophilus</i> NCFB1748	Lidbeck 1988 <sup>54</sup>	5	culture	healthy volunteers	enoxacin or	1	restores only in enoxacin	restores only in enoxacin,
		5	culture	volunteers	clindamycin	1	no change	no change in clindamycin
<i>L. rhamnosus</i> GG	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>S. boulardii</i> lyo	Zoppi 2001 <sup>51</sup>	6	culture	pediatric respiratory infections	ceftriaxone	0	improves	partially restores
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	Black 1991, <sup>55</sup> Zoppi 2001 <sup>51</sup>	10,	culture,	healthy volunteers,	ampicillin,	2,	recovers more rapidly,	restores,
		7	culture	pediatric respiratory	ceftriaxone	0	less change	partially restores
<i>L. acidophilus</i> 1748 + <i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	no change	no change
<i>L. rhamnosus</i> + <i>L. bifidus</i> + <i>L. acidophilus</i>	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido lactis</i> Bb12	Jernberg 2005 <sup>56</sup>	4	culture PCR TRFLP	healthy volunteers	clindamycin	2	restores	restores
<i>L. acidophilus</i> CUL60+ <i>L. acidophilus</i> CUL21 + <i>Bifido. bifidum</i> CUL17 + <i>Bifido animalis lactis</i>	Madden 2005, <sup>57</sup> Plummer 2005 <sup>58</sup>	15,	culture,	<i>H. pylori</i> +,	amoxicillin + metronidazole,	2,	restores,	restores,
		76	culture	<i>H. pylori</i> +	amoxicillin + clarithromycin	2	restores more rapidly	partially restores
<i>L. acidophilus</i> NCFM + <i>L. paracasei</i> Lpc-37 + <i>Bifido. bifidum</i> Bb02+ <i>Bifido. lactis</i> Bi-04 + <i>Bifido. lactis</i> Bi-07	Engelbrektsen 2006 <sup>50</sup>	20	culture PCR TRFLP	healthy volunteers	augmentin	2	restores	restores

\*including strain (when reported)

**Table 2. Model B:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 18 probiotics from 24 studies (25 treatment arms) with disturbed microbiota at baseline.

Probiotic*	Reference	No. treated with probiotic	Type(s) of assay for NM	Pre-existing disrupting factor**	Follow-up time	Claims stated in papers	Evidence-based claim	Type of change found in NM
<i>Bifido. breve</i> M-16V	Van der Aa 2010 <sup>59</sup>	46	FISH	Atopic dermatitis	0	modulates NF	no change	--
<i>Bifido. lactis</i> Bi-07	Larsen 2011 <sup>53</sup>	17	PCR	Atopic dermatitis	0	no change	no change	--
<i>Bifido. longum</i> BB536	Odamaki 2007 <sup>33</sup>	22	TRFLP PCR	Cedar pollen allergy	4 wk	maintains NF	no change	--
<i>E. coli</i> Nissle	Lata 2007 <sup>60</sup>	22	culture	liver cirrhosis	0	restores	improves	more <i>Bifido.</i> & <i>Lacto.</i>
<i>L. acidophilus</i> 700396	Larsen 2011 <sup>53</sup>	17	PCR	atopic dermatitis	0	no change	no change	--
<i>L. casei rhamnosus</i> Lcr35	Petricevic 2008 <sup>61</sup>	83	Nugent scores	bacterial vaginosis	4 wk	restores	improves	improved Nugent scores
<i>L. plantarum</i> 299v	Nobaek 2000, <sup>62</sup>	25,	culture,	IBS,	4 wk,	no change,	no change,	--
	Klarin 2005, <sup>63</sup>	17,	culture,	enterally-fed,	0,	no change,	no change,	
	Klarin 2008 <sup>64</sup>	22	culture	antibiotics	0	no change	no change	
<i>S. boulardii</i> Iyo	Girard 2002, <sup>65</sup>	10,	culture,	enterally-fed,	9 d,	alters NF,	no change,	--
	Swidsinski 2008 <sup>66</sup>	20	FISH	active diarrhea	3 wk	improves	improves	more 'habitual microbiota'
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2001 <sup>67</sup>	33	Nugent scores	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2003 <sup>68</sup>	31	Nugent scores and culture	bacterial vaginosis	30 d	restores	improves	improved Nugent scores
<i>L. plantarum</i> 8PA3 + <i>Bifido bifidum</i>	Kirpich 2008 <sup>69</sup>	32	culture	colon cancer	0	restores	improves	more <i>E. coli</i> and <i>Enterococci</i>
<i>L. rhamnosus</i> GR1 + <i>L. reuteri</i> RC14	Hummelen 2010 <sup>70</sup>	23	Nugent score	bacterial vaginosis	0	no change	no change	--
<i>L. casei</i> Shirota+ <i>Bifido breve</i> BBG01	Uchida 2007 <sup>71</sup>	4	culture	short bowel syndrome	0	no change	no change	--
<i>L. brevis</i> CD2 + <i>L. salivaris</i> FV2 + <i>L. plantarum</i> FV9	Mastromarino 2009 <sup>72</sup>	19	Nugent score	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores

<i>L. paracasei</i> Lpc37+ <i>L. acidophilus</i> 74-2 + <i>Bifido. animalis</i> DGCC420	Roessler 2012 <sup>73</sup>	30	PCR	atopic dermatitis	0	no change	no change	--
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii</i> shermanii JS + <i>Bifido. breve</i> Bb99	Kajander 2005, <sup>74</sup>	41,	PCR,	IBS,	0,	restores,	improves,	Improved similarity index
	Lyra 2010 <sup>75</sup>	22	PCR	IBS	0	alters	alters	More Clostridia and Rumino-coccus
<i>L. acidophilus</i> 4356 + <i>L. plantarum</i> 14917 + <i>L. rhamnosus</i> 7469 + <i>Bifido. bifidum</i> 2952	Wong 2013 <sup>76</sup>	7	PCR	liver disease	0	improves	alters	Less Firmicutes, more Bacteroidetes
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Venturi 1999, <sup>77</sup>	20,	culture	ulcerative colitis,	15 d,	enhances,	no change,	--
	Brigidi 2001, <sup>78</sup>	10,	culture & PCR	IBS,	10 d,	no change,	no change,	--
	Kuhbacher 2006 <sup>79</sup>	10	FISH	pouchitis	0	altered richness	altered	More anaerobes
	Ng 2013 <sup>80</sup>	10	PCR	IBS	0	modulates	altered	Less Bacteroides

\*including strain (when reported)

\*\*disruption of normal microbiota at baseline shown by significant differences compared to control (non-diseased) population.

**Table 3. Model C:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 19 probiotics in healthy volunteers enrolled in 29 studies (29 treatment arms) in studies with no disruptive exposures.

Probiotic	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment	Claims stated in papers	Evidence-based claim
<i>Bifido. animalis lactis</i> DN173010	Rochet 2008, <sup>49</sup> Oswari 2013 <sup>81</sup>	12, 160	FISH PCR	healthy volunteers	none, none	10 d, 6 mon	no change, no change	no change, no change
<i>Bifido. bifidum</i>	Langhendries 1995 <sup>82</sup>	20	culture	healthy volunteers	none	0	no change	no change
<i>Bifido. longum</i>	Benno 1992, <sup>83</sup> Fujiwara 2001, <sup>84</sup> Harmsen 2002 <sup>85</sup>	5, 7, 14	culture, culture, FISH	healthy volunteers	none, none, none	0, 30 d, 0	no change, alters, no change	no change, alters, no change
<i>L. casei</i> ND114001	Guerin 1998, <sup>86</sup> Rochet 2006, <sup>87</sup> Rochet 2008 <sup>88</sup>	12, 12, 7	culture, FISH, FISH	healthy volunteers	none, none, none	1 wk, 10 d, 0	no change, no change, no change	no change, no change, no change
<i>L. johnsonii</i> Lal	Brunser 2006 <sup>89</sup>	32	culture & FISH	healthy volunteers	none	2 wk	no claim	no change
<i>L. plantarum</i> 299v	Goossens 2003, <sup>90</sup> Goossens 2005, <sup>91</sup> Goossens 2006, <sup>92</sup> Berggren 2003, <sup>93</sup> Karlsson 2010 <sup>94</sup>	11, 32, 15, 33, 9	culture, culture, culture, TRFLP	healthy, healthy, colonic polyps, healthy, atherosclerosis	none, none, none, none, none	3 wk, 4 wk, 0, 0, 0	no change, no change, no change, no change, alters	no change, no change, no change, no change, alters
<i>L. rhamnosus</i> GG	Gueimonde 2006 <sup>95</sup>	29	PCR	healthy volunteers	none	0	no change	no change
<i>L. salivarius</i> CECT5713	Sierra 2010 <sup>96</sup>	20	culture	healthy volunteers	none	0	improves	no change
<i>S. boulardii</i> lyo	Vanhoutte 2006 <sup>97</sup>	30	PCR	healthy volunteers	none	0	no change	no change
<i>Bifido. animalis</i> + <i>Bifido. longum</i>	Zhong 2006, <sup>98</sup> He 2008 <sup>99</sup>	11, 11	FISH, FISH	healthy volunteers	none	7 d, 7d	no change, modifies	no change, no change
<i>L. acidophilic</i> + <i>Bifido. lactis</i>	Yang 2012 <sup>100</sup>	63	culture	healthy but 55% <i>H. pylori</i> +	none	0	restores	alters
<i>L. rhamnosus</i> GG + <i>Bifido. longum</i> Bb536	Mah 2007 <sup>101</sup>	20	FISH	healthy neonates	none	6 mon	no change	no change
<i>L. rhamnosus</i> GG + <i>Bifido. lactis</i> Bb12	Rafter 2007 <sup>102</sup>	38	culture	colon cancer patients or at risk	none	0	no change	no change

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36	<i>L. rhamnosus</i> GG + <i>L. gasseri</i> TMC0356	Kubota 2009 <sup>103</sup>	14	culture FISH TRFLP	healthy, allergy patients	none	0	suppressed changes	alters
	<i>L. paracasei</i> B21060 + <i>L. paracasei</i> B21070 + <i>L. gasseri</i> B21090	Morelli 2003 <sup>104</sup>	12	culture	healthy volunteers	none	3 d	no claims	no change
	<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	Sullivan 2009 <sup>105</sup>	15	culture	chronic fatigue patients	none	4 wk	no change	no change
	<i>L. rhamnosus</i> 271 + <i>L. acidophilus</i> NCFM + <i>L. paracasei</i> 114001 + <i>Bifido. animalis</i> 1017	Engelbrektson 2006 <sup>50</sup>	22	culture TRFLP PCR	healthy volunteers	none	2 wk	no change	no change
	<i>Bifido. animalis lactis</i> + <i>L. delbrueckii</i> I-1632 + <i>L. delbrueckii</i> I-1519 + <i>L. lactis cremoris</i>	McNulty 2011 <sup>106</sup>	7	PCR	healthy twins volunteers	none	4 wk	no change	no change
	<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp. bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Vitali 2012 <sup>14</sup>	15	PCR	healthy pregnant volunteers	none	0	modulates	no change

37 \*including strain (when reported)

38 Abbreviations: FISH, fluorescence *in situ* hybridization analysis; TRFLP, terminal restriction fragment  
39 length polymorphism analysis; PCR, polymerase chain reaction  
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**Table 4.** Comparison of the ability of probiotic to restore or improve dysbiosis with ranked clinical efficacy for various disease indications.

Probiotic*	Restored normal microbiota *	Altered normal microbiota*	Ranked net evidence for efficacy**							
			AAD	CDI	IBD	IBS	TD	H pylori	Vaginitis/BV	Acute Ped diar
<b>Restores microbiota</b>										
<i>Clostr. butyricum</i> MIYAIRI	yes	nd	-						-	
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	yes	nd	0	-						
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	yes	nd				-				
<i>Bifido. longum</i>	yes	no			-	+				
<i>L. acidophilus</i> + <i>L. acidophilus</i> + <i>Bifido. bifidum</i> + <i>Bifido. animalis</i>	yes	nd								
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i> (2)	yes	no								
<i>S. boulardii</i> lyo	partial	yes	++	++	++	0	+	-		++
<i>L. rhamnosus</i> GG	partial	nd	-	-	-	0	0	-	0	++
<i>L. acidophilus</i>	partial	no	++			++	-	-	+	0
<i>L. acidophilus</i> + <i>L. bifidus</i> + <i>L. rhamnosus</i>	partial	nd								
<b>Alters microbiota</b>										
<i>E. coli</i> Nissle	nd	yes			-					+
<i>L. casei</i> (DN114001 or Lcr35)	nd	yes	+					0	+	++
<i>L. rhamnosus</i> GR1 + <i>L. fermentum</i> RC14	nd	yes							++	
<i>L. plantarum</i> 8PA3 + <i>Bifido. bifidum</i>	nd	yes								
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii</i> <i>shermanii</i> JS + <i>Bifido. breve</i> Bb99	nd	yes				++				
<i>L. acidophilus</i> + <i>L. plantarum</i> + <i>L. rhamnosus</i> + <i>Bifido</i> <i>bifidum</i>	nd-	yes								



<i>L. brevis</i> CD2 + <i>L. salivarius</i> FV2 + <i>L. plantarum</i> FV9	nd	yes							+	
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> + <i>L. plantarum</i> , <i>Bifido.</i> <i>longum</i> , <i>Bifido.</i> <i>infantis</i> , <i>Bifido.</i> <i>breve</i>	nd	yes	-		++	+				++
<b>No effect on microbiota</b>										
<i>B. clausii</i>	nd	nd						-		-
<i>L. plantarum</i> 299v	nd	no	-	-		-				
<i>Bifido. lactis</i>	nd	no	+							0
<i>Bifido. breve</i>	no	no								
<i>L. acidophilus</i> + <i>Bifido. longum</i>	no	--								
<i>L. rhamnosus</i> 19070-2 + <i>L. reuteri</i> DSM	nd	no								0
<i>L. casei</i> + <i>Bifido. breve</i>	nd	no								
<i>L. paracasei</i> + <i>L. acidophilus</i> + <i>Bifido animalis</i>	nd	no								
<b>Pharmacokinetic only</b>										
<i>L. reuteri</i> 55730	nd	nd								+
<i>L. johnsonii</i> La1	nd	nd			-			+		
<i>L. salivarius</i> UCC4331	nd	nd				-				
<i>Bifido. infantis</i> 35624	nd	nd				0				
<i>Bifido. bifidum</i> MIMBb75	nd	nd				+				
<i>L. rhamnosus</i> + <i>Bifido. longum</i>	nd	nd								

\*including strain (when reported)

\*\* **Rank:** ++,  $\geq 2$  net RCTs (randomized controlled trials) with significant protective efficacy; +, only one net protective RCT; 0, equal number of significant and non-significant RCTs; -,  $\geq 1$  net non-significant RCT. Blank indicates no RCT done for the disease indication.

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3 **Abbreviations:** nd, not determined; AAD, antibiotic associated diarrhea; CDI, Clostridium  
4 difficile infections; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; TD,  
5 traveler's diarrhea; BV, bacterial vaginosis; Acute Ped Diar, treatment of acute pediatric  
6 diarrhea.  
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For peer review only

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16 **Figure 1.** Time sequence of events and three models of study designs determining three different  
17 degrees of dysbiosis correction by probiotics.  
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**Figure 2.** Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota.

For peer review only

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5 **Use of probiotics to correct dysbiosis of normal microbiota following disease**  
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8 **or disruptive events: a systematic review.**  
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27 **Disclaimer:** *The findings and conclusions in this study are those of the author  
28 and do not represent the official position of the University.*

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37 work(s) based on the Contribution, iv) to exploit all subsidiary rights in the Contribution,  
38 v) the inclusion of electronic links from the Contribution to third party material where-  
39 ever it may be located; and vi) licence any third part to do any or all of the above.  
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44 **Word Count** 6731

45 **Figures:** 2

46 **Tables:** 4  
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## ABSTRACT

**Objective:** To assess the evidence for the claim probiotics can correct dysbiosis of the normal microbiota resulting from disease or disruptive events.

**Setting:** Systematic review of published clinical trials of patients receiving a probiotic intervention for the prevention or treatment of various diseases.

**Data sources:** Sources searched (1985-2013): PubMed, EMBASE, Cochrane Database of Systematic Reviews, CINAHL, AMED, and ISI Web of Science. Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.

**Review methods:** Included studies were randomized clinical trials of probiotic interventions having microbiologic assays. Studies were evaluated following PRISMA guidelines for specific probiotic strains. A standard data extraction form was used to collect the raw data.

**Outcome measures:** The primary outcome is the degree of microbiota correction by specific probiotic strains. Secondary outcome was the association between the degree of dysbiosis correction and clinical efficacy.

**Results:** The review of the literature found three distinct study designs: Model A (restoration) assayed patients enrolled with a healthy, undisturbed microbiota and then assayed post-disruptive event and probiotic therapy; Model B (alteration) assayed patients with pre-existing disrupted microbiota and then post-probiotic therapy; Model C (no dysbiosis) assayed volunteers with no disruptive event pre and post-probiotic. From a total of 63 trials, 83% of the probiotic products using Model A restored the microbiota, 56% using Model B improved the microbiota and only 21% using Model C had any effect on microbiota. Clinical efficacy was more commonly associated with strains capable of restoration of the normal microbiota.

**Conclusions:** The ability to assess the degree of dysbiosis improvement is dependent upon the enrolled population and the timing of microbiologic assays. The functional claim for correcting dysbiosis is poorly supported for most probiotic strains and requires further research.

**Systematic review registration:** PROSPERO (CRD42014007224)

## Strengths and Limitations

### Strengths include:

- A comprehensive review of the published literature from 1985-2013
- Literature search unrestricted by language or country
- Analysis of study designs resulted in novel strategy to limit bias and classify outcomes
- Three types of outcomes of dysbiosis applied to evidence-based studies of specific probiotic strains
- Author has over 40 years of research experience in the probiotic field

### Limitations include:

- Pooled clinical trials using different study populations
- Pooled probiotic doses and regimens
- Indirect evidence linking probiotic strains and dysbiosis
- Review done by sole author

## INTRODUCTION

The popularity of probiotics has expanded exponentially recently, but along with their increased use, debate rages on how probiotics should be regulated and whether probiotics should be considered as a medical food, drug or a food supplement. In the U.S., probiotics are typically available as dietary supplements and thus are limited to 'structure or function' health claims and, unlike prescription drugs, are not permitted to claim to 'treat' or 'cure' disease. In Europe and the United Kingdom, probiotics are allowed to have health or function claims. These claims are required to be supported by well-conducted human trials in the targeted population or in healthy volunteers, but the European Food Safety Authority (EFSA) has rejected >80% of claims submitted to them.<sup>1-3</sup> In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect.<sup>4</sup> One such functional claim made for probiotic products is they correct dysbiosis (or the disruption of bacterial and fungal species after antibiotics or other disruptive exposures) and thus may be beneficial to maintain health. Probiotics are active during this susceptible window from the time of the disruptive event to the time when normal microbiota is restored. A wide variety of mechanisms-of-action have been documented for probiotics (ranging from blocking pathogen attachment sites, destruction of the pathogen by bacteriocins or proteases that degrade toxins, to regulation of the immune system),<sup>5,6</sup> and while clinical evidence supports efficacy of some probiotic strains, the evidence linking these mechanisms-of-action to a specific health or function claims is not as clear.

A classic example of the consequence of dysbiosis is antibiotic-associated diarrhea (AAD).<sup>7,8</sup> While antibiotics may be effective in the elimination of pathogenic organisms, a common, unintended effect is the killing or inhibition of beneficial microbes due to shared susceptibility to the antibiotic. One of the many functions for normal microbiota is the ability to resist infection by pathogenic organisms, termed 'colonization resistance'.<sup>9,10</sup> The loss of a sub-population of the normal microbiota, for example, can lead to the loss of the ability to break down fibers and starches into absorbable short chain fatty acids, resulting in high level of undigested carbohydrates, which can trigger diarrhea.<sup>11</sup> Disruption of the normal microbiota has been shown to lead to higher rates of infections in other body systems other than the intestinal tract including the skin,<sup>12,13</sup> vagina,<sup>14,15</sup> respiratory tract,<sup>16,17</sup> and in the buccal cavity.<sup>18-20</sup>

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6 The major challenge to establishing a cause and effect for the improvement of dysbiosis by  
7 probiotics is a lack of a standard definition of ‘normal’ microbiota. There is substantial inter-  
8 individual variation of the species of microbes present at different body niches, which also varies  
9 by age, geographic area and health status of the host. In addition, a complete accounting of the  
10 microbiota is currently impossible, as there are no assays to detect all of  $>10^{13}$ - $10^{14}$  organisms in  
11 the intestines and standard microbial culturing methods miss 75-95% of these organisms.<sup>21,22</sup>  
12 The development of metagenomics (cataloguing individual and disease-specific bacterial gene  
13 profiles) and the creation of the international Human Microbiome Project ushered in a new era  
14 for our understanding of the complexity of these interactions within the body.<sup>23,24</sup> This paradigm  
15 shift from culturing to metagenomic analysis has expanded our ability to document shifts in  
16 microbial populations to an unparalleled degree, but the interpretation of these shifts continues to  
17 be under debate.<sup>25-28</sup> With the advent of these newer metagenomic tools, the role of probiotics in  
18 the restoration of normal microbiota is being re-visited.<sup>29</sup>  
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32 In light of new guidance documents and recommendations, the goal of this systematic review is  
33 to determine how claims for the restoration of the normal microbiota and the correction of  
34 dysbiosis have been studied using well-designed trials and which probiotic strains have  
35 evidence-based data to support these claims.  
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## 42 **METHODS**

### 43 **Study Objective**

44 To systematically review the literature to analyse the evidence for the claim probiotics can  
45 correct dysbiosis of the normal microbiota from randomised controlled trials.  
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### 52 **Search Strategy**

53 Search terms included: probiotics + health claims, restoring normal microbiota, dysbiosis,  
54 normal microbiota, pharmacokinetics, metagenomics, probiotics, dietary supplements,  
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3 randomized controlled trials, antibiotic associated diarrhea (AAD), *Clostridium difficile* infection  
4 (CDI), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), traveler's diarrhea  
5 (TD), eradication of *Helicobacter pylori*, bacterial vaginosis or vaginitis, treatment of acute  
6 pediatric diarrhea, and specific probiotic strains or products. Search strategies were broad-based  
7 initially, then narrowed to clinical trials with probiotics.  
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### 13 14 **Data Sources**

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16 PubMed (1985-2013), EMBASE (1985-2013), Cochrane Database of Systematic Reviews  
17 (1990-2013), CINAHL (1985-2013), AMED (1985-2013), and ISI Web of Science (2000-2013).  
18 Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled  
19 trials (<http://www.cochrane.org>), MetaRegister of Controlled Trials (<http://www.controlled->  
20 [trials.com/mrct](http://www.controlled-trials.com/mrct)) and National Institutes of Health (<http://www.clinicaltrials.gov>).  
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### 27 **Criteria for study selection and data extraction**

28 Abstracts of all citations were reviewed by a single author and rated for inclusion for randomized  
29 controlled trials of probiotic treatments. Full articles were retrieved if normal microbiota assays  
30 were mentioned. Non-English language trials were translated and included whenever possible.  
31 Exclusion criteria included pre-clinical studies (animal models or *in vitro* assays), safety or phase  
32 2 studies, reviews, efficacy trials with no assays for normal microbiota species, metagenomic  
33 methods only, mechanism of action of normal microbiota or probiotic, cross-sectional surveys,  
34 case reports or case series, duplicate reports, or trials of unspecified types of probiotics. All  
35 pharmacokinetic studies in humans were reviewed, as abstracts often did not include normal  
36 microbiota assay data. Data extraction and the review process followed the PRISMA statement  
37 guidelines using a 27-item checklist and flow diagram.<sup>30</sup> A standardized data extraction form  
38 was used to collect data on the probiotic (strain type, daily dose, duration), type of controls  
39 (placebo, active or no treatment), study design (status of microbiota at baseline and follow-up  
40 times), type of microbiota assay (microbial culturing, molecular biomarkers, etc.), enrolled study  
41 population (adult vs. pediatric, healthy volunteers, disease condition), type and timing of  
42 disruptive agent (antibiotics, chemotherapy, etc.), study size and attrition, outcome assessment  
43 (efficacy and/or microbiota status at end of study, adverse events) and type of health claim.  
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## Outcomes and definitions

The primary outcome is the degree of microbiota correction or improvement by specific probiotic strain(s). The secondary outcome is the association between the degree of dysbiosis correction and the net efficacy found from randomized controlled trials of probiotic interventions. Dysbiosis is defined as an alteration or disruption of the normal microbiota (bacterial or fungal species) due to exposure of a disruptive factor (such as antibiotics, chronic disease, stress, medical procedures or medications, etc.). As there is no current standard definition of 'normal' microbiota, for this review, restoration of normal microbiota is defined as a return to the assayed microbial species or profile taken from a healthy individual (before a disruptive event has occurred). Included studies are required to have at least a pre-probiotic treatment assay and a post-probiotic treatment assay. A variety of microbial assays were available during the search period (1985-2013), including documentation of the microbiota by either microbial cultures, or metagenomic methods [16s rRNA-targeted probes using fluorescent *in situ* hybridization (FISH) or other polymerase chain reaction (PCR) technique]<sup>8,21,28,31</sup> or by indirect methods (Nugent scores).<sup>15</sup> Nugent scores (ranged 0-10) are used to diagnose bacterial vaginosis (scores  $\geq 7$ ) or normal vaginal microbiota (scores 0-3) based on the quantitated morphotypes of small gram negative rods (*G. vaginalis/Bacteroides* spp.) and curved gram negative rods (*Mobiluncus* spp.) from gram stains of vaginal discharge smears. Microbial assays of only the strain(s) contained in the probiotic product are considered as pharmacokinetic studies and were not included in the normal microbiota profiles.

**Models of dysbiosis.** To determine the impact on normal microbiota, only direct evidence of microbiota change (species, profiles, diversity indices, or diagnostic criteria) were included and indirect effects were excluded (changes in intestinal enzymes, immune system parameters or disease symptoms). The degree to which dysbiosis was improved is categorized into three levels: (1) recovery of the normal microbiota back to baseline levels; (2) alteration or improvement of the normal microbiota; and (3) no change in normal microbiota.

The literature contained three dysbiosis models: Model A (restoration of the normal microbiota), which assayed patients enrolled with a healthy, undisturbed microbiota and then assayed again after a disruptive event (such as antibiotic exposure) and probiotic therapy occurred; Model B (alteration of the microbiota) assayed patients with pre-existing disrupted microbiota (for

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3 example, pre-existing chronic disease or active disease) and then post-probiotic therapy; Model  
4 C (no dysbiosis) assayed volunteers with no disruptive event (before or during the clinical trial)  
5 at both pre-probiotic and post-probiotic times, as shown in Figure 1. 'Recovery' of the normal  
6 microbiota is defined as a restoration of the microbiota back to a normal healthy baseline.  
7 Recovery may be complete recovery (all assayed microbial levels returned to baseline) or  
8 incomplete recovery (partial recovery of some microbial strains, but not all returned to baseline  
9 levels). In studies enrolling subjects with dysbiosis at baseline (typically due to chronic  
10 diseases), it is not possible to show a restoration to normal microbiota levels because a normal,  
11 undisturbed microbiota was not present in these types of study subjects at the time of enrollment.  
12 Therefore, the strongest claim possible for Model B designs is for an 'alteration or improvement'  
13 of the microbiota. Only data from the probiotic-exposed subjects were analysed in this paper.  
14 Data from the control groups were used to confirm dysbiosis for subjects with chronic diseases  
15 or after a disruptive exposure, such as antibiotics or chemotherapy, unaffected by probiotic  
16 exposure.<sup>32-34</sup>  
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### 30 **Assessment of methodological strength and quality**

31 The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system  
32 for rating overall study quality will be used for each probiotic strain or type (single strains and  
33 mixtures of strains).<sup>35</sup> Recommendation for the support of the claim of each probiotic strain or  
34 mixture can be assessed by the overall strength of the evidence ["strong", many randomized  
35 controlled trials show significant recovery of the microbiota, or "moderate" only one randomized  
36 controlled trial; or "weak", only case series or reports, limited number of small trials, *etc.*].  
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44 Quality of the evidence is based on study design and graded as "high quality" (well-defined  
45 study design for determining restoration with normal microbiota, Model A), or "moderate  
46 quality" (disrupted microbiota at baseline, Model B), or "low quality" (no disruptive event  
47 occurred, Model C). Measurement of publication bias was not assessed for this review, as  
48 pooled outcome estimates of efficacy were not done, as typical in meta-analysis, but all studies  
49 with assays of microbiota were included to limit bias.  
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### 56 **Net efficacy rating**

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3 To determine if the ability to correct dysbiosis is associated with clinical efficacy, the published  
4 literature for randomized controlled trials (RCTs) or meta-analyses of probiotics for various  
5 disease indications, including antibiotic associated diarrhea (AAD),<sup>5,36,37</sup> *Clostridium difficile*  
6 infection (CDI),<sup>5,38</sup> inflammatory bowel disease (IBD),<sup>39</sup> irritable bowel syndrome (IBS),<sup>40</sup>  
7 traveler's diarrhea (TD),<sup>41</sup> eradication of *Helicobacter pylori* (Hp),<sup>36,37</sup> bacterial vaginosis (BV)  
8 or vaginitis,<sup>42</sup> and treatment of acute pediatric diarrhea was reviewed.<sup>43-45</sup> The net rank was  
9 calculated by subtracting the number of RCTs showing non-significant or equivalent efficacy  
10 from the number of RCTs having significant efficacies. The ranks were categorized as follows:  
11 ++,  $\geq 2$  net RCTs showing significant efficacy; +, net of one RCT showing significant efficacy; 0,  
12 equal number of RCTs showing significant and non-significant efficacy results and -,  $\geq 1$  net  
13 negative or non-significant RCTs. Probiotics with no RCTs were not ranked.  
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## 25 RESULTS

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27 A review of the literature from 1985-2013 found 353 articles that dealt with probiotic treatments  
28 and their potential effect on normal microbiota.  
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### 33 Excluded studies

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35 As shown in Figure 2, a total of 272 articles were excluded for the following reasons: reviews  
36 (n=116), probiotic efficacy studies with no data on normal microbiota assays (n=54), animal  
37 models of probiotics and changes in microbiota (n=38), metagenomic or microbiota methods  
38 only (n=17), studies on normal microbiota but with no use of probiotics (n=14), *in vitro* assays of  
39 microbiota (n=10), duplicative reports (n=2) or miscellaneous (n=21), which included probiotic  
40 mechanism of action studies, safety studies, duplicative reports, cross-sectional surveys and two  
41 with poorly described probiotic interventions.<sup>46,47</sup> A total of 81 full articles were reviewed which  
42 mentioned changes in normal microbiota or indicated a health claim for probiotics and effects on  
43 normal microbiota.  
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52 Probiotic pharmacokinetic studies (n=18) reporting concentrations of probiotic strains before and  
53 post-treatment, but did not assaying for other species of normal microbiota were excluded.  
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55 While several studies using this study design claim probiotics had an impact on normal  
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3 microbiota, type of data generated is pharmacokinetic behavior of the probiotics themselves and  
4 not the normal microbiota. Several studies stated that the normal microbiota was altered because  
5 an increase in various bacterial species was observed after the probiotics were given, but the  
6 species assayed were those contained in the probiotic product, so an increase is not unexpected.  
7 Pharmacokinetic studies have documented that probiotic strains taken orally can survive transit  
8 through the intestinal tract with recovery rates in feces ranging from <1% to 22%.<sup>48,49</sup> These  
9 pharmacokinetic studies were excluded from this analysis, as they did not assay other types of  
10 normal microbiota not found in the probiotic product.  
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### 20 **Included studies**

21 Of the 63 included clinical trials, five trials had multiple treatment arms, which resulted in a total  
22 of 68 treatment arms for analysis. Engelbrekton et al. tested a mixture of 5 probiotic strains in  
23 volunteers exposed to antibiotics and also tested a mixture of 4 probiotic strains in healthy  
24 volunteers with no antibiotic exposure.<sup>50</sup> Zoppi et al. had eight different treatment arms in his  
25 study, and probiotic arms were included in our analysis [*Saccharomyces boulardii* (*S. boulardii*)  
26 alone and *Lactobacillus* (*L.*) *rhamnosus* GG alone], a mixture of two probiotics (*L. acidophilus*  
27 and *Bifido. bifidum*) and a mixture of three probiotic strains (*L. acidophilus*, *L. rhamnosus* and  
28 *Bifido. bifidum*).<sup>51</sup> Orrhage et al. had two treatment arms (*Bifido. longum* alone and a mixture of  
29 *Bifido. longum* and *L. acidophilus*).<sup>52</sup> Larsen et al. tested two single probiotics (*Bifido. lactis* and  
30 *L. acidophilus*) in separate treatment arms.<sup>53</sup> Lidbeck et al. gave either enoxacin or clindamycin  
31 and randomized patients to either *L. acidophilus* or placebo.<sup>54</sup>  
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44 **Normal microbiota assay methods.** Of the 69 treatment arms that did normal microbiota assays,  
45 diverse methods were used to profile the microbiota. Many studies used only standard  
46 microbiological culture assays (37, 54%), while others (28, 40%) used techniques to detect non-  
47 cultivatable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16s rRNA  
48 sequencing) or other PCR techniques. Some studies (4, 6%) used an indirect measure of normal  
49 microbiota, using the Nugent score to diagnose bacterial vaginosis, which relies upon gram stain  
50 of the vaginal secretions, vaginal pH and symptoms to characterize if normal microbiota is  
51 present or absent.<sup>15</sup>  
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6 **Probiotic strains.** In the 69 treatment arms, most (36, 52%) used a single strain of probiotic,  
7 while 14 (20%) tested a mix of two probiotic strains and 19 (28%) tested a mix of three or more  
8 probiotic strains. The distribution of single versus multiple strain probiotics did not significant  
9 vary by the model of study design ( $\chi^2_2=2.3$ , P=0.32). Of the 15 restorative (Model A) study  
10 arms, 47% used a single strain of probiotic and 53% used multiple strains. Of the 25 treatment  
11 arms with disrupted microbiota at baseline (Model B), 44% used a single strain and 56% used  
12 multiple strains. Of the 29 study arms with undisturbed microbiota (Model C), 62% used a  
13 single strain and 38% used multiple strains.  
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### 23 **Normal microbiota restoration model (Model A)**

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25 Only 10 studies (with 15 treatment arms) using Model A to determine restoration of the  
26 microbiota were found (Table 1).<sup>32,34,50-52,54-58</sup> The type of enrolled subjects varied from healthy  
27 volunteers to children with untreated respiratory infections, to pediatric cancer patients. For  
28 subjects with acute infections or cancer, baseline assays were done prior to the disrupting agent  
29 (antibiotics or chemotherapy). The number of subjects given probiotics averaged 20/study and  
30 ranged from 5 to 83. In 93%, the disruptive factor was antibiotic exposure and in one study,  
31 chemotherapy caused the microbiota disruption. Only 8 (53%) of the study arms did an assay  
32 during a 1-8 week follow-up period after the probiotic was discontinued.  
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43 Analysis of the probiotic strain(s) separately found only two probiotic products with more than  
44 one randomized controlled trial. The probiotic mix of *L. acidophilus* and *Bifido. bifidum* showed  
45 a complete restoration in one study, but only a partial recovery in the other. (Strength: strong,  
46 Quality: high). The probiotic mix of *L. acidophilus* (2 strains) with *Bifido. bifidum* and *Bifido.*  
47 *animalis* showed complete restoration in one study, but only a partial recovery in the other.  
48 (Strength: strong, Quality: high). Five other probiotic products with only one supporting clinical  
49 trial showed microbiota restoration (*Bifido. longum*, *Clostridium butyricum*, *L. acidophilus*, mix  
50 of *L. acidophilus* with *L. paracasei* and *Bifido. lactis*, and the mix of *L. acidophilus* with *L.*  
51 *paracasei* and *Bifido. bifidum* and two strains of *Bifido. lactis*). (Strength: moderate, Quality:  
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3 high). Three probiotic products with one supporting clinical trial showed partial restoration (*S.*  
4 *boulevardii*, *L. rhamnosus* GG, mix of *L. rhamnosus* with *L. bifidus* and *L. acidophilus*), (Strength:  
5 moderate, Quality: high). Only two probiotic products using Model A showed no change in the  
6 microbiota (*Bifido. breve* and a mix of *L. acidophilus* and *Bifido. longum*). (Strength: moderate,  
7 Quality: high). In summary, 10 of 12 (83%) of the probiotic products showed complete or partial  
8 restoration of the normal microbiota.  
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18 Of the 11 probiotic products with claims of 'restores or improves normal microbiota', 10 (91%)  
19 were supported by this review, but only seven showed complete restoration and five had partial  
20 restoration of the microbiota (Table 1). The mixture of *L. acidophilus* and *Bifido. longum* did  
21 not show any changes in the microbiota. Wada et al. claimed *Bifido. breve* 'enhanced intestinal  
22 anaerobes', but this was only compared to the placebo group.<sup>32</sup> Their data showed chemotherapy  
23 is a disruptive event, resulting in more Enterobacteria in the intestine in the placebo group, but  
24 there were no significant differences seen by the end of the 8 week follow-up in either the  
25 probiotic or the placebo group compared to baseline microbiota levels.  
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### 35 **Disrupted normal microbiota at baseline studies (Model B)**

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37 Twenty-four studies (with 25 treatment arms) used Model B that enrolled subjects with a pre-  
38 existing disrupted microbiota related to ongoing disease or conditions (Table 2).<sup>33,53,59-80</sup> The  
39 number of subjects given probiotics averaged  $23 \pm 16$ /study and ranged from 7-83 participants.  
40 The types of pre-existing factors that disrupted the microbiota included atopic dermatitis  
41 patients, allergies, cirrhosis, bacterial vaginosis, irritable bowel syndrome, inflammatory bowel  
42 disease (ulcerative colitis and pouchitis), idiopathic diarrhea, enteral feeding, short-bowel  
43 syndrome and colon cancer. Only 10 (40%) of the study arms did an assay during the post-  
44 probiotic follow-up period.  
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55 Three of the probiotics had multiple clinical trials to support the claim of an improvement in the  
56 microbiota due to the probiotic. *S. boulevardii* was used in two trials either with enteral fed  
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3 patients or patients with active diarrhea and found an improvement in the habitual microbiota in  
4 the patients with active diarrhea<sup>66</sup>, but only showed indirect evidence of short-chain fatty acid  
5 changes in the other study.<sup>65</sup> (Strength: strong, Quality: moderate) A mix of four probiotic  
6 strains (2 strains of *L. rhamnosus*, *P. freudenreichii* + *Bifido. breve*) showed improved  
7 microbiota in two clinical trials.<sup>74,75</sup> (Strength: strong, Quality: moderate) Of four clinical trials  
8 testing a mixture of seven probiotic strains, two showed no significant change in microbiota<sup>77,78</sup>,  
9 one showed more anaerobes post-probiotic treatment<sup>79</sup> and one found a reduction in *Bacteroides*  
10 species.<sup>80</sup> (Strength: strong, Quality: moderate) Three clinical trials determined there were no  
11 significant changes due to *L. plantarum* 299v.<sup>62-64</sup> (Strength: strong, Quality: moderate). Of those  
12 probiotics with only one supporting clinical trial (Strength: moderate, Quality: moderate), two  
13 single probiotic strains (*E. coli* Nissle and *L. casei rhamnosus*) and five different mixtures of  
14 probiotic strains support the claim that the probiotic alters the microbiota (Table 2). In summary,  
15 10 of 18 (56%) probiotic products altered or improved microbiota in individuals with pre-  
16 existing disease.  
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32 Of the 25 treatment arms, the paper's claim was confirmed in 14 (56%) of the studies. There was  
33 no significant change in the microbiota due to the probiotic in nine treatment arms and only an  
34 alteration of the microbiota in five others (Table 2). Our review disagreed with the claimed  
35 outcomes in 11 (46%) of the other treatment arms. In seven treatment arms, it was claimed the  
36 tested probiotic 'restored normal microbiota', but it is uncertain how this conclusion was reached,  
37 since there was no time when a normal undisrupted microbiota was present. Of the seven studies  
38 that claimed their probiotic 'restored' normal microbiota, our analysis determined none were  
39 capable of documenting restoration, but it is confirmed probiotics improved or altered the  
40 microbiota in these studies. Four studies claimed the probiotic 'altered or improved' normal  
41 microbiota, but this review found no significant differences when post-probiotic and baseline  
42 assays were compared for the probiotic groups. Girard-Pipau et al. concluded that *S. boulardii*  
43 'altered normal flora' because more gram positive anaerobes were seen in the probiotic group  
44 compared to the controls and an increase in three short-chain fatty acids were observed in the *S.*  
45 *boulardii* group.<sup>65</sup> However, when the analysis is restricted to trends observed in the probiotic  
46 group only, no significant differences were observed in pre-probiotic versus post-probiotic  
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3 microbiota profiles. Venturi et al. concluded that the mix of seven probiotic strains enhanced the  
4 concentration of some beneficial strains in the intestines.<sup>77</sup> However, the only strains having a  
5 significant increase were those contained in the probiotic mix, and not specifically normal  
6 microbiota of the host. As this study did not have an undisturbed microbiota baseline, the  
7 increased numbers of Lactobacilli and Bifidobacteria may not have reflected their normal levels.  
8 Van der Aa et al. claimed that *Bifido. breve* 'successfully modulates the intestinal flora', but no  
9 significant changes were observed in the probiotic group when comparing the baseline to the  
10 post-probiotic levels.<sup>59</sup> Odamaki et al. did show an increase in Faecalibacterium ssp. and  
11 *Bacteroides fragilis* ssp. at the end of *Bifido. longum* BB536 treatment, but the same increase  
12 was also observed in the placebo group.<sup>33</sup>  
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#### 24 **Undisrupted normal microbiota studies (Model C)**

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27 Twenty nine trials enrolled healthy adults who had no disruptive factor present during the study  
28 (either no antibiotic or no medication exposure or presence of acute or chronic disease) that  
29 might impact normal microbiota, as shown in Table 3.<sup>14,49,50,81-106</sup> The average number of  
30 subjects given probiotics was 23/study and ranged from 7 to 160/study. Of the 29 study arms,  
31 assays were taken during a follow-up period in only 52%. Fujiwara et al. cultured seven healthy  
32 volunteers and found Enterobacteriaceae and Clostridial species post-*Bifido. longum* was  
33 reduced by 10<sup>1</sup>/g compared to baseline (P<0.03), but no other changes in the microbiota were  
34 detected.<sup>84</sup> Karlsson et al. found a significant increase in intestinal diversity in nine male  
35 volunteers with atherosclerosis given *L. plantarum* 299v, but because terminal restriction  
36 fragment length polymorphism assays were used instead of cultures for bacterial species, the  
37 specific changes in the microbiota species could not be determined.<sup>94</sup> Yang and Sheu cultured 63  
38 children (55% with *H. pylori*) given a yogurt with *L. acidophilus* and *Bifido. lactis* but only  
39 found a decrease in *E. coli* counts in the *H. pylori* negative children sub-group, no significant  
40 changes in normal microbiota was found in the *H. pylori* positive children.<sup>100</sup> Kubota et al.  
41 assayed 29 subjects with Japanese cedar pollen allergy and found milk fermented with *L.*  
42 *rhamnosus* GG and *L. gasseri* TMC0356 suppressed microbiota changes (less intestinal profile  
43 changes), but could not determine specific bacterial species changes due to the type of assay used  
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3 (FISH and TRFLP).<sup>103</sup> In summary, only 4 of 19 (21%) probiotic products altered microbiota in  
4 healthy individuals who had no disruptive event.  
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10 Of the seven studies that claimed their probiotic(s) 'restored or altered' the normal microbiota,  
11 only four claims were confirmed. Sierra et al. claimed *L. salivarius* given to 20 healthy adults  
12 'improved gut microbiota', but only increased levels of Lactobacilli were found and no other  
13 changes in normal microbiota species were detected. The only other evidence was indirect from  
14 changes observed in immune parameters.<sup>96</sup> He et al. claimed a mixture of *Bifido. longum* and  
15 *Bifido. animalis* 'modified' microbiota, but changes were seen only during the yogurt  
16 administration and not after the one week follow-up period.<sup>99</sup> Vitali et al. claimed that the  
17 mixture of four Lactobacilli strains and three Bifidobacteria strains 'modulated vaginal  
18 microbiota', but the only significant changes were due to an increase in the bacterial species  
19 contained in the probiotic mixture.<sup>14</sup>  
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31 Of the probiotics supported by multiple clinical trials (*Bifido. animalis*, *Bifido. longum*, *L. casei*,  
32 *L. plantarum* 299v, the mixture of *Bifido. animalis* and *Bifido. lactis*), 13 of the trials (87%)  
33 support there is no significant change in normal microbiota if the microbiota is not disrupted.  
34 [Strength: strong, Quality: low]  
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#### 42 **Association of clinical efficacy and normal microbiota restoration**

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44 Few studies concurrently compared clinical efficacy and the ability to restore or improve normal  
45 microbiota after dysbiosis. A synthesis of the literature of RCT for eight common disease  
46 indications was performed and the overall net strength was ranked. Probiotics with the ability to  
47 restore normal microbiota were frequently supported by RCTs for efficacy, as shown in Table 4.  
48 Of the 10 probiotics with evidence for restoration, 7 (70%) also had at least one RCT testing for  
49 at least one of the eight diseases, while 30% did not have any supportive RCTs for efficacy. Of  
50 the 7 probiotics with associated RCTs, only two probiotics (*S. boulardii* and *L. acidophilus*) have  
51 strong evidence for efficacy across most of the disease indications, while five probiotics with the  
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3 ability to restore the microbiota had weak or no evidence of efficacy. For example, *S. boulardii*,  
4 which has studies supporting restoration, has strong evidence for clinical efficacy for AAD  
5 (ranked ++: 11 RCTs had significant results and 6 had non-significant results), CDI (ranked ++:  
6 had two RCTs with significant results), IBD (ranked ++: had two RCTs with significant results),  
7 IBS (ranked 0: had one RCT with significant efficacy and one RCT with non-significant results),  
8 TD (ranked +: 3 RCTs with significant efficacy and 2 with non-significant efficacy), *H. pylori*  
9 eradication (ranked -: 2 RCTs with significant results and 4 with non-significant results) and no  
10 studies for BV. *L. acidophilus*, which partially restored the microbiota in a study, is associated  
11 with clinical efficacy for AAD, IBS and BV, but not for TD or eradication of *H. pylori* and  
12 treatment of acute pediatric diarrhea (ranked ++: had 19 RCTs with significant protection and  
13 five with non-significant results). In contrast, *L. rhamnosus GG*, another probiotic capable of  
14 restoring microbiota, is often cited in meta-analysis as having significant efficacy for AAD. Our  
15 results of an updated review of the literature indicate a net weak evidence rating for clinical  
16 efficacy across all disease indications: AAD (ranked -: 3 RCTs had significant results and 6 had  
17 non-significant results), CDI (ranked -: two RCTs with non-significant results), IBD (ranked -:  
18 one RCT with non-significant results), IBS (ranked 0: 2 RCTs with significant efficacy and two  
19 RCTs with non-significant results), TD (ranked 0: one RCT with significant efficacy and one  
20 with non-significant efficacy), *H. pylori* eradication (ranked -: 3 RCTs with non-significant  
21 results), no RCTs for BV and treatment of acute pediatric diarrhea (ranked ++: 10 RCTs with  
22 significant efficacy and one with non-significant findings).  
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42 Efficacy trials were not done as frequently for probiotics shown to only have the ability to alter  
43 or improve, but not restore, the microbiota after dysbiosis. Of nine probiotics that can alter the  
44 microbiota, 6 (67%) have supporting RCTs for at least one disease, but the diversity of  
45 investigated diseases was more limited. *L. casei* had moderate net strength for AAD and  
46 bacterial vaginosis, but was neutral for the ability to eradicate *H. pylori* and other disease  
47 indications were not tested in RCTs with *L. casei*. The probiotic mixture of *L. reuteri* and *L.*  
48 *fermentum* has strong evidence for bacterial vaginosis, but not for any other disease indications  
49 listed in Table 4.  
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Of the eight probiotics not capable of altering or restoring normal microbiota, only *L. plantarum* 299v had RCTs for AAD and IBS, both with net negative or weak strength of clinical efficacy. *Bifido. lactis* and the mixture of *L. rhamnosus* and *L. reuteri* had net neutral rankings for efficacy for the treatment of acute pediatric diarrhea. The other four probiotic products with no effect on normal microbiota lacked any RCTs for clinical efficacy. Studies with *B. clausii* did not assay for normal microbiota and had non-significant trial results for *H. pylori* eradication and the treatment of pediatric diarrhea.

Of the six probiotics with only pharmacokinetic data on the probiotic itself and no other investigation of other normal microbiota strains, five had RCTs showing varying net efficacies for different disease indications, as shown in Table 4.

Six popular probiotics (*Bacillus clausii*, *Bifido. infantis*, *L. reuteri*, *L. acidophilus* + *L. helveticus*, *L. acidophilus* + *L. casei* and *L. acidophilus* + *Bifido. animalis*) have only clinical efficacy RCTs, but have not published studies investigating their role in restoring or improving the normal microbiota.

## Discussion

Developing and evaluating health or function claims for probiotics is an important issue and is now identified as a priority for research by several international organizations, including the World Gastroenterology Organization<sup>107</sup> and the American Society for Nutrition.<sup>2</sup> The U.S. Food and Drug Administration has struggled with appropriate evidence-based health claims for probiotic products and currently recommends the use of structure/function claims, such as "maintains bowel regularity", but the claim for restoring normal microbiota is still under debate.<sup>108</sup> The European Food Safety Authority (EFSA) provides guidance materials that recommend health or function claims for probiotics should have beneficial physiological effects and have appropriate scientific trials to substantiate the health claims.<sup>3</sup> Acceptable claims for intestinal health may include functional claims (improved transit time, softer stool consistency, reduction in gastrointestinal discomfort, defense against pathogens). As it is currently not possible to define a standard normal microbiota profile, the EFSA recommends functional claims



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3 for the restoration of normal microbiota should document a recovery of healthy microbiota and  
4 be accompanied by a beneficial physiological or clinical outcome.<sup>3</sup> In addition, because the  
5 efficacy and mechanisms are strain-specific and may vary by probiotic strain, the evidence must  
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7 be analyzed for each probiotic product individually.<sup>5,6,9,109, 110-112</sup>  
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12 An underappreciated finding was the influence that study design and study populations have on  
13 the interpretation of study outcomes. In the literature, five different types of study designs are  
14 commonly found relating to probiotics. The most common study type is a randomized controlled  
15 trial testing the efficacy and safety outcomes in patients, but these trials did not typically  
16 document the impact of the probiotic on the normal microbiota. The second most common type  
17 of study design is pharmacokinetic studies (documenting recovery of oral dose of probiotic or  
18 increase in probiotic strains post-treatment compared to pre-treatment or clearance of the  
19 probiotic). Even though these kinetic studies did not assay for non-probiotic strains, some  
20 extrapolated their results and concluded some effect or improvement of the normal microbiota  
21 was observed by their probiotic.<sup>19,111</sup> These two first types of study designs do not support  
22 evidence-based conclusions for the restoration or alteration of the normal microbiota and were  
23 excluded from this review.  
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34 Three types of study designs are appropriate for the study of dysbiosis. The first type of study  
35 design had normal microbiota assayed at least twice (at baseline, which was before exposure to a  
36 disruptive event or probiotics and then again during or post-probiotic treatment) to show actual  
37 recovery of assayed normal microbiota back to healthy baseline levels. The second type of study  
38 design started with inappropriate baselines (baseline samples taken after normal microbiota had  
39 been disrupted by chronic disease). For patients with established chronic diseases, there is no  
40 “normal microbiota” baseline in either the probiotic or the control group. Even if baselines are  
41 taken during remission, the microbiota may still be impacted by chronic disease or acute  
42 diarrhea. Studies of probiotics in chronic diseases or acute disease typically report on ‘pre-  
43 probiotic treatment’ and ‘post-probiotic treatment’ and may show significant shifts in microbial  
44 species, but it is uncertain if this reflects a true re-establishment of normal microbiota profiles.  
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46 The third type of study design enrolled healthy volunteers, who were not challenged with  
47 antibiotics (so no normal microbiota disruption occurred), and show only the effect of probiotics  
48 on a healthy microbiota (typically mild or no effects). Control groups were not required for our  
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3 assessment of the impact of probiotics on microbiota, but control groups can document the  
4 degree normal microbiota is disrupted by inciting agents (antibiotic, disease onset, etc.).  
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10 Five single strain probiotics (*Bifido. longum*, *Clost. butyricum*, *L. acidophilus*, *L. rhamnosus* and  
11 *S. boulardii*) and five probiotic mixtures [(*L. acidophilus* + *Bifido. bifidum*), (*L. rhamnosus* + *L.*  
12 *bifidus* + *L. acidophilus*), (*L. acidophilus* + *L. paracasei* + *Bifido. lactis*), (*L. acidophilus*, 2  
13 strains, *Bifido. bifidum*, *Bifido. animalis*) and (*L. acidophilus* + *L. paracasei* + *Bifido. bifidum*  
14 + 2 strains of *Bifido. lactis*)] documented either complete or partial recovery of normal  
15 microbiota (Model A). Only two probiotic mixtures [(2 strain mixture: *L. acidophilus* + *Bifido.*  
16 *bifidum*) and (4 strain mixture: *L. acidophilus*, 2 strains, *Bifido. bifidum*, *Bifido. animalis*)] were  
17 supported by a confirmatory study. Evidence that probiotics may alter or improve normal  
18 microbiota (Model B) was found for three single strain probiotics (*E. coli* Nissle, *S. boulardii*  
19 and *L. casei rhamnosus*) and seven mixtures of 2-7 probiotic strains. Of these ten probiotics  
20 finding alteration of the microbiota, only three had multiple trials [*S. boulardii*, and a four strain  
21 mixture (2 strains of *L. rhamnosus* + *P. freudenreichii* + *Bifido. breve*), and a seven strain  
22 mixture (4 Lactobacilli and 3 Bifidobacteria strains)], but only one had consistent results  
23 showing improvements in the microbiota.<sup>74,75</sup> Clearly, more than one study is needed to confirm  
24 the impact of a probiotic on the normal microbiota. Of the 19 probiotic strains (or mixtures)  
25 studied in healthy volunteers who were not exposed to disruptive factors (Model C), no change  
26 in the normal microbiota was observed for 79%, indicating the robustness of the microbiota.  
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44 Improvement in the normal microbiota by specific probiotic strains seemed to be associated with  
45 better clinical endpoints. Within eight common diseases typically treated with probiotics, more  
46 trials with significant efficacy were associated with probiotic strains shown to restore the normal  
47 microbiota, and only one trial with significant efficacy was found for probiotics that did not alter  
48 the microbiota. However, few probiotics had efficacy trials for all eight diseases and many did  
49 not have any efficacy trials.  
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3 Some probiotics which have published efficacy trials for various diseases did not have studies  
4 investigating the effect of the probiotic on normal microbiota: *Bacillus clausii*, *Bifido. infantis*, *L.*  
5 *brevis*, *L. reuteri*, mix of 2 strains (*L. acidophilus* + *L. helveticus*), mix of 2 strains (*L.*  
6 *acidophilus* + *L. casei*) or (*L. acidophilus* + *Bifido. animalis*), mix of 4 strains [*L. rhamnosus* (2  
7 strains), *Propionibacterium freudenreichii* + *Bifido. animalis*] and mix of 7 strains (*L.*  
8 *sporogens*, *L. bifidum*, *L. bulgaricus*, *L. thermophilus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*).

### 17 **Comparison of results with other studies**

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20 Other reviews in the literature of claims for probiotics relating to changes in the normal  
21 microbiota have focused on the broad issues of regulatory standardization of health or function  
22 claims, the use of proper study designs and the challenge of defining biomarkers for a 'healthy  
23 microbiota'.<sup>3,29,112</sup> Donovan et al. recommends that health claims for probiotics be supported by  
24 well-conducted human trials in the targeted population.<sup>2</sup> These reviews also recommend that gut  
25 biomarkers need to be correlated with clinical endpoints, however none of these reviews  
26 attempted to do so.<sup>29,112</sup> No prior review has attempted to analyze the association between  
27 probiotic strains and their impact on normal microbiota by stratifying on the quality of study  
28 design.<sup>111</sup> This review addressed these concerns by analyzing probiotic strains by the quality of  
29 the study design and only including trials that assessed the normal microbiota (either by  
30 microbial culturing or molecular strain biomarkers) and assessed the degree of dysbiosis  
31 improvement with clinical outcomes for each probiotic strain.

### 45 **Opportunities for future research**

47 Most of the studies (80%) using Model A to document restoration of the normal microbiota only  
48 used microbiologic culturing techniques, which can only detect those organisms that grow in  
49 culture. Use of the more advanced molecular metagenomic techniques have found that culturing  
50 alone misses up to 95% of these organisms.<sup>21,22</sup> The use of the metagenomic techniques was  
51 more common in the studies using Model B (48%) and Model C (45%) study designs, which  
52 only addresses potential alteration of the microbiota. Characterization of the microbiota is a  
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3 complex issue and a comprehensive accounting of all the bacterial and fungal strains in the body  
4 is beyond our current capabilities. Therefore, any studies of changes to the microbiota are  
5 incomplete at best, but general trends in bacterial phylotypes can be documented using DNA  
6 probes and metagenomic techniques. Differential detection bias may be present due to the  
7 variety of assays used in these studies and should be accounted for in future studies.  
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16 Another suggestion for future studies is to include an appropriate follow-up time period post-  
17 probiotic administration. Fewer than half of the reviewed trials did assays for normal microbiota  
18 during an appropriate follow-up period. As it has been shown that recovery from a disrupting  
19 factor can be prolonged (typically eight weeks),<sup>7,8</sup> and studies that failed to find microbiota  
20 recovery might have detected a return to normal baseline levels if a sufficiently long time was  
21 given for the recovery to have occurred. Future studies should strive to allow time for the  
22 restoration of the normal microbiota to occur.  
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31 As the effects of probiotics are strain specific, and many studies typically only report the genus  
32 and species of the tested probiotic, future reports should include a complete description of the  
33 probiotic to the strain level.<sup>5,112</sup>  
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### 39 **Strengths and weaknesses**

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42 The strengths of this review included the completeness of the search strategy, which reviewed  
43 multiple citation databases, trial registries and author searches, use of established PRISMA  
44 protocols for reviews and the use of an outcome classification scheme for different degrees of  
45 assessment for microbial recovery. This analysis controlled the confounding effects of different  
46 study populations and study designs present in the literature. Pharmacokinetic studies of just the  
47 probiotic strain(s) itself were excluded and only trials that assayed other species found in the  
48 microbiota were included. By applying a standard definition for 'restoring' versus 'improving'  
49 normal microbiota, it is possible to distinguish significant differences by the type of study  
50 designs used and differential effects of the different probiotic strains. Limitations of this review  
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3 include: a single author reviewed and extracted the literature, pooling trials from different  
4 populations (adult versus pediatric) and different probiotic doses and regimens used. Incomplete  
5 retrieval of all studies assessing the effect that probiotics have on human microbiota is also a  
6 potential limitation of any literature search. Another limitation is that dysbiosis improvement and  
7 clinical efficacy for probiotic strains is also indirectly associated, no direct cause and effect  
8 relationship was possible with the types of studies done. Another limitation is the current lack of  
9 a standard definition of what comprises a 'normal microbiota'. The constituents of the  
10 microbiota vary by individual, by age, geographic location and health status of the host. Current  
11 microbiologic techniques are improving, but can not detect all species present in the host.  
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## 22 **Conclusion**

23 The challenges in recommending a specific probiotic to patients who need to restore or improve  
24 their normal microbiota after a disrupting event occurs is two-fold: one is the diversity of  
25 probiotic products available and second is the varying strength of evidence provided by clinical  
26 trials using different outcome measures and study designs. By grouping studies into three groups  
27 that result in three different degrees of probiotic effect (restoration, improvement or no change),  
28 an overview of the body of evidence is possible. By comparing the strength of the clinical  
29 evidence for common diseases by the degree to which the probiotics could impact the restoration  
30 of the normal microbiota, it became obvious that those probiotics with a greater ability to restore  
31 the microbiota are associated with the strongest strength of clinical efficacy. While this evidence  
32 only indirectly links clinical efficacy with the ability to restore the microbiota, the overall review  
33 of the evidence shows this is an important mechanism of action for probiotics. What becomes  
34 obvious is that more studies are required to conclude which probiotic strains have a beneficial  
35 impact on the normal microbiota, as most strains have only a single clinical trial and many  
36 probiotic products overstate the strength of their claim to restore normal microbiota. These types  
37 of issues should be considered for health care policy makers and researchers for future studies  
38 and for creating guidelines for health/function claims.  
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**Table 1. Model A:** Evidence-based data for restoration of normal microbiota (NM) for 12 probiotics from 10 studies (15 treatment arms).

Probiotic*	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment (wks)	Claims stated in papers	Evidence-based claim
<i>Bifido. breve</i>	Wada 2010 <sup>32</sup>	19	FISH	pediatric cancer patients	chemotherapy	8	enhances anaerobes	no change
<i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	restores	restores
<i>Clost. butyricum</i> MIYAIRI	Seki 2003 <sup>34</sup>	83	culture	pediatric respiratory or GI infections	antibiotics	0	restores	restores
<i>L. acidophilus</i> NCFB1748	Lidbeck 1988 <sup>54</sup>	5	culture	healthy volunteers	enoxacin or	1	restores only in enoxacin	restores only in enoxacin,
		5	culture	volunteers	clindamycin	1	no change	no change in clindamycin
<i>L. rhamnosus</i> GG	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>S. boulardii</i> lyo	Zoppi 2001 <sup>51</sup>	6	culture	pediatric respiratory infections	ceftriaxone	0	improves	partially restores
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	Black 1991, <sup>55</sup> Zoppi 2001 <sup>51</sup>	10,	culture,	healthy volunteers,	ampicillin,	2,	recovers more rapidly,	restores,
		7	culture	pediatric respiratory	ceftriaxone	0	less change	partially restores
<i>L. acidophilus</i> 1748 + <i>Bifido. longum</i> BB536	Orrhage 1994 <sup>52</sup>	10	culture	healthy volunteers	clindamycin	0	no change	no change
<i>L. rhamnosus</i> + <i>L. bifidus</i> + <i>L. acidophilus</i>	Zoppi 2001 <sup>51</sup>	7	culture	pediatric respiratory infections	ceftriaxone	0	partially corrects	partially restores
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido lactis</i> Bb12	Jernberg 2005 <sup>56</sup>	4	culture PCR TRFLP	healthy volunteers	clindamycin	2	restores	restores
<i>L. acidophilus</i> CUL60+ <i>L. acidophilus</i> CUL21 + <i>Bifido. bifidum</i> CUL17 + <i>Bifido animalis lactis</i>	Madden 2005, <sup>57</sup> Plummer 2005 <sup>58</sup>	15,	culture,	<i>H. pylori</i> +,	amoxicillin + metronidazole,	2,	restores,	restores,
		76	culture	<i>H. pylori</i> +	amoxicillin + clarithromycin	2	restores more rapidly	partially restores
<i>L. acidophilus</i> NCFM + <i>L. paracasei</i> Lpc-37 + <i>Bifido. bifidum</i> Bb02+ <i>Bifido. lactis</i> Bi-04 + <i>Bifido. lactis</i> Bi-07	Engelbrektsen 2006 <sup>50</sup>	20	culture PCR TRFLP	healthy volunteers	augmentin	2	restores	restores

\*including strain (when reported)

**Table 2. Model B:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 18 probiotics from 24 studies (25 treatment arms) with disturbed microbiota at baseline.

Probiotic*	Reference	No. treated with probiotic	Type(s) of assay for NM	Pre-existing disrupting factor**	Follow-up time	Claims stated in papers	Evidence-based claim	Type of change found in NM
<i>Bifido. breve</i> M-16V	Van der Aa 2010 <sup>59</sup>	46	FISH	Atopic dermatitis	0	modulates NF	no change	--
<i>Bifido. lactis</i> Bi-07	Larsen 2011 <sup>53</sup>	17	PCR	Atopic dermatitis	0	no change	no change	--
<i>Bifido. longum</i> BB536	Odamaki 2007 <sup>33</sup>	22	TRFLP PCR	Cedar pollen allergy	4 wk	maintains NF	no change	--
<i>E. coli</i> Nissle	Lata 2007 <sup>60</sup>	22	culture	liver cirrhosis	0	restores	improves	more Bifido. & Lacto.
<i>L. acidophilus</i> 700396	Larsen 2011 <sup>53</sup>	17	PCR	atopic dermatitis	0	no change	no change	--
<i>L. casei rhamnosus</i> Lcr35	Petricevic 2008 <sup>61</sup>	83	Nugent scores	bacterial vaginosis	4 wk	restores	improves	improved Nugent scores
<i>L. plantarum</i> 299v	Nobaek 2000, <sup>62</sup>	25,	culture,	IBS,	4 wk,	no change,	no change,	--
	Klarin 2005, <sup>63</sup>	17,	culture,	enterally-fed,	0,	no change,	no change,	
	Klarin 2008 <sup>64</sup>	22	culture	antibiotics	0	no change,	no change,	
<i>S. boulardii</i> Iyo	Girard 2002, <sup>65</sup>	10,	culture,	enterally-fed,	9 d,	alters NF,	no change,	--
	Swidsinski 2008 <sup>66</sup>	20	FISH	active diarrhea	3 wk	improves	improves	more 'habitual microbiota'
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2001 <sup>67</sup>	33	Nugent scores	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores
<i>L. rhamnosus</i> GR-1 + <i>L. fermentum</i> RC14	Reid 2003 <sup>68</sup>	31	Nugent scores and culture	bacterial vaginosis	30 d	restores	improves	improved Nugent scores
<i>L. plantarum</i> 8PA3 + <i>Bifido bifidum</i>	Kirpich 2008 <sup>69</sup>	32	culture	colon cancer	0	restores	improves	more <i>E. coli</i> and <i>Enterococci</i>
<i>L. rhamnosus</i> GR1 + <i>L. reuteri</i> RC14	Hummelen 2010 <sup>70</sup>	23	Nugent score	bacterial vaginosis	0	no change	no change	--
<i>L. casei</i> Shirota+ <i>Bifido breve</i> BBG01	Uchida 2007 <sup>71</sup>	4	culture	short bowel syndrome	0	no change	no change	--
<i>L. brevis</i> CD2 + <i>L. salivaris</i> FV2 + <i>L. plantarum</i> FV9	Mastromarino 2009 <sup>72</sup>	19	Nugent score	bacterial vaginosis	2 wk	restores	improves	improved Nugent scores

<i>L. paracasei</i> Lpc37+ <i>L. acidophilus</i> 74-2 + <i>Bifido. animalis</i> DGCC420	Roessler 2012 <sup>73</sup>	30	PCR	atopic dermatitis	0	no change	no change	--
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii shermanii</i> JS + <i>Bifido. breve</i> Bb99	Kajander 2005, <sup>74</sup>	41,	PCR,	IBS,	0,	restores,	improves,	Improved similarity index
	Lyra 2010 <sup>75</sup>	22	PCR	IBS	0	alters	alters	More Clostridia and Rumino-coccus
<i>L. acidophilus</i> 4356 + <i>L. plantarum</i> 14917 + <i>L. rhamnosus</i> 7469 + <i>Bifido. bifidum</i> 2952	Wong 2013 <sup>76</sup>	7	PCR	liver disease	0	improves	alters	Less Firmicutes, more Bacteroidetes
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp. bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Venturi 1999, <sup>77</sup>	20,	culture	ulcerative colitis,	15 d,	enhances,	no change,	--
	Brigidi 2001, <sup>78</sup>	10,	culture & PCR	IBS,	10 d,	no change,	no change,	--
	Kuhbacher 2006 <sup>79</sup>	10	FISH	pouchitis	0	altered richness	altered	More anaerobes
	Ng 2013 <sup>80</sup>	10	PCR	IBS	0	modulates	altered	Less Bacteroides

\*including strain (when reported)

\*\*disruption of normal microbiota at baseline shown by significant differences compared to control (non-diseased) population.



**Table 3. Model C:** Evidence-based data for improvement or alteration of normal microbiota (NM) in 19 probiotics in healthy volunteers enrolled in 29 studies (29 treatment arms) in studies with no disruptive exposures.

Probiotic	Reference	No. treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment	Claims stated in papers	Evidence-based claim
<i>Bifido. animalis lactis</i> DN173010	Rochet 2008, <sup>49</sup> Oswari 2013 <sup>81</sup>	12, 160	FISH PCR	healthy volunteers	none, none	10 d, 6 mon	no change, no change	no change, no change
<i>Bifido. bifidum</i>	Langhendries 1995 <sup>82</sup>	20	culture	healthy volunteers	none	0	no change	no change
<i>Bifido. longum</i>	Benno 1992, <sup>83</sup> Fujiwara 2001, <sup>84</sup> Harmsen 2002 <sup>85</sup>	5, 7, 14	culture, culture, FISH	healthy volunteers	none, none, none	0, 30 d, 0	no change, alters, no change	no change, alters, no change
<i>L. casei</i> ND114001	Guerin 1998, <sup>86</sup> Rochet 2006, <sup>87</sup> Rochet 2008 <sup>88</sup>	12, 12, 7	culture, FISH, FISH	healthy volunteers	none, none, none	1 wk, 10 d, 0	no change, no change, no change	no change, no change, no change
<i>L. johnsonii</i> Lal	Brunser 2006 <sup>89</sup>	32	culture & FISH	healthy volunteers	none	2 wk	no claim	no change
<i>L. plantarum</i> 299v	Goossens 2003, <sup>90</sup> Goossens 2005, <sup>91</sup> Goossens 2006, <sup>92</sup> Berggren 2003, <sup>93</sup> Karlsson 2010 <sup>94</sup>	11, 32, 15, 33, 9	culture, culture, culture, culture, TRFLP	healthy, healthy, colonic polyps, healthy, atherosclerosis	none, none, none, none, none	3 wk, 4 wk, 0, 0, 0	no change, no change, no change, no change, alters	no change, no change, no change, no change, alters
<i>L. rhamnosus</i> GG	Gueimonde 2006 <sup>95</sup>	29	PCR	healthy volunteers	none	0	no change	no change
<i>L. salivarius</i> CECT5713	Sierra 2010 <sup>96</sup>	20	culture	healthy volunteers	none	0	improves	no change
<i>S. boulardii</i> lyo	Vanhoutte 2006 <sup>97</sup>	30	PCR	healthy volunteers	none	0	no change	no change
<i>Bifido. animalis</i> + <i>Bifido. longum</i>	Zhong 2006, <sup>98</sup> He 2008 <sup>99</sup>	11, 11	FISH, FISH	healthy volunteers	none	7 d, 7d	no change, modifies	no change, no change
<i>L. acidophilic</i> + <i>Bifido. lactis</i>	Yang 2012 <sup>100</sup>	63	culture	healthy but 55% <i>H. pylori</i> +	none	0	restores	alters
<i>L. rhamnosus</i> GG + <i>Bifido. longum</i> Bb536	Mah 2007 <sup>101</sup>	20	FISH	healthy neonates	none	6 mon	no change	no change
<i>L. rhamnosus</i> GG + <i>Bifido. lactis</i> Bb12	Rafter 2007 <sup>102</sup>	38	culture	colon cancer patients or at risk	none	0	no change	no change

<i>L. rhamnosus</i> GG + <i>L. gasseri</i> TMC0356	Kubota 2009 <sup>103</sup>	14	culture FISH TRFLP	healthy, allergy patients	none	0	suppressed changes	alters
<i>L. paracasei</i> B21060 + <i>L. paracasei</i> B21070 + <i>L. gasseri</i> B21090	Morelli 2003 <sup>104</sup>	12	culture	healthy volunteers	none	3 d	no claims	no change
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	Sullivan 2009 <sup>105</sup>	15	culture	chronic fatigue patients	none	4 wk	no change	no change
<i>L. rhamnosus</i> 271 + <i>L. acidophilus</i> NCFM + <i>L. paracasei</i> 114001 + <i>Bifido. animalis</i> 1017	Engelbrektsen 2006 <sup>50</sup>	22	culture TRFLP PCR	healthy volunteers	none	2 wk	no change	no change
<i>Bifido. animalis lactis</i> + <i>L. delbrueckii</i> I-1632 + <i>L. delbrueckii</i> I-1519 + <i>L. lactis cremoris</i>	McNulty 2011 <sup>106</sup>	7	PCR	healthy twins volunteers	none	4 wk	no change	no change
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp. bulgaricus</i> + <i>L. plantarum</i> + <i>Bifido. longum</i> + <i>Bifido. infantis</i> + <i>Bifido. breve</i>	Vitali 2012 <sup>14</sup>	15	PCR	healthy pregnant volunteers	none	0	modulates	no change

\*including strain (when reported)

Abbreviations: FISH, fluorescence *in situ* hybridization analysis; TRFLP, terminal restriction fragment length polymorphism analysis; PCR, polymerase chain reaction

**Table 4.** Comparison of the ability of probiotic to restore or improve dysbiosis with ranked clinical efficacy for various disease indications.

Probiotic*	Restored normal microbiota *	Altered normal microbiota*	Ranked net evidence for efficacy**							
			AAD	CDI	IBD	IBS	TD	H pylori	Vaginitis/BV	Acute Ped diar
<b>Restores microbiota</b>										
<i>Clostr. butyricum</i> <i>MIYAIRI</i>	yes	nd	-						-	
<i>L. acidophilus</i> + <i>Bifido. bifidum</i>	yes	nd	0	-						
<i>L. acidophilus</i> 1748 + <i>L. paracasei</i> F19 + <i>Bifido. lactis</i> Bb12	yes	nd				-				
<i>Bifido. longum</i>	yes	no			-	+				
<i>L. acidophilus</i> + <i>L. acidophilus</i> + <i>Bifido. bifidum</i> + <i>Bifido. animalis</i>	yes	nd								
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>Bifido. lactis</i> (2)	yes	no								
<i>S. boulardii lyo</i>	partial	yes	++	++	++	0	+	-		++
<i>L. rhamnosus GG</i>	partial	nd	-	-	-	0	0	-	0	++
<i>L. acidophilus</i>	partial	no	++			++	-	-	+	0
<i>L. acidophilus</i> + <i>L. bifidus</i> + <i>L. rhamnosus</i>	partial	nd								
<b>Alters microbiota</b>										
<i>E. coli</i> Nissle	nd	yes			-					+
<i>L. casei</i> (DN114001 or Lcr35)	nd	yes	+					0	+	++
<i>L. rhamnosus</i> GR1 + <i>L. fermentum</i> RC14	nd	yes							++	
<i>L. plantarum</i> 8PA3 + <i>Bifido. bifidum</i>	nd	yes								
<i>L. rhamnosus</i> GG + <i>L. rhamnosus</i> Lc705 + <i>P. freudenreichii</i> <i>shermanii</i> JS + <i>Bifido. breve</i> Bb99	nd	yes				++				
<i>L. acidophilus</i> + <i>L. plantarum</i> + <i>L. rhamnosus</i> + <i>Bifido</i> <i>bifidum</i>	nd-	yes								

<i>L. brevis</i> CD2 + <i>L. salivarius</i> FV2 + <i>L. plantarum</i> FV9	nd	yes							+	
<i>L. acidophilus</i> + <i>L. paracasei</i> + <i>L. delbrueckii ssp.</i> <i>bulgaricus</i> + <i>L. plantarum</i> , <i>Bifido.</i> <i>longum</i> , <i>Bifido.</i> <i>infantis</i> , <i>Bifido.</i> <i>breve</i>	nd	yes	-		++	+				++
<b>No effect on microbiota</b>										
<i>B. clausii</i>	nd	nd						-		-
<i>L. plantarum</i> 299v	nd	no	-	-		-				
<i>Bifido. lactis</i>	nd	no	+							0
<i>Bifido. breve</i>	no	no								
<i>L. acidophilus</i> + <i>Bifido. longum</i>	no	--								
<i>L. rhamnosus</i> 19070-2 + <i>L. reuteri</i> DSM	nd	no								0
<i>L. casei</i> + <i>Bifido. breve</i>	nd	no								
<i>L. paracasei</i> + <i>L. acidophilus</i> + <i>Bifido animalis</i>	nd	no								
<b>Pharmacokinetic only</b>										
<i>L. reuteri</i> 55730	nd	nd								+
<i>L. johnsonii</i> La1	nd	nd			-			+		
<i>L. salivarius</i> UCC4331	nd	nd				-				
<i>Bifido. infantis</i> 35624	nd	nd				0				
<i>Bifido. bifidum</i> MIMBb75	nd	nd				+				
<i>L. rhamnosus</i> + <i>Bifido. longum</i>	nd	nd								

\*including strain (when reported)

\*\* **Rank:** ++,  $\geq 2$  net RCTs (randomized controlled trials) with significant protective efficacy; +, only one net protective RCT; 0, equal number of significant and non-significant RCTs; -,  $\geq 1$  net non-significant RCT. Blank indicates no RCT done for the disease indication.

**Abbreviations:** nd, not determined; AAD, antibiotic associated diarrhea; CDI, Clostridium difficile infections; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; TD, traveler’s diarrhea; BV, bacterial vaginosis; Acute Ped Diar, treatment of acute pediatric diarrhea.

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6 **Contributorship statement:** The listed author made substantial contributions to the conception  
7 and design, acquisition of data, analysis and interpretation of the data, drafting the article and  
8 revising it critically for important intellectual content and had final approval of the version to be  
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10 preparation. This manuscript (in full or in part) is not under consideration for publication in any  
11 other journal, nor is a duplicative paper.  
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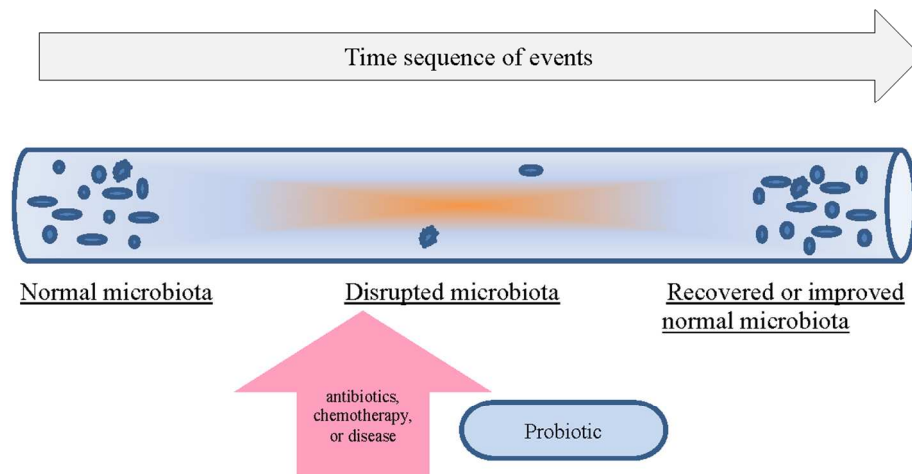
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16 **Figure 1.** Time sequence of events and three models of study designs determining three different  
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18 degrees of dysbiosis correction by probiotics.  
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**Figure 2.** Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota.

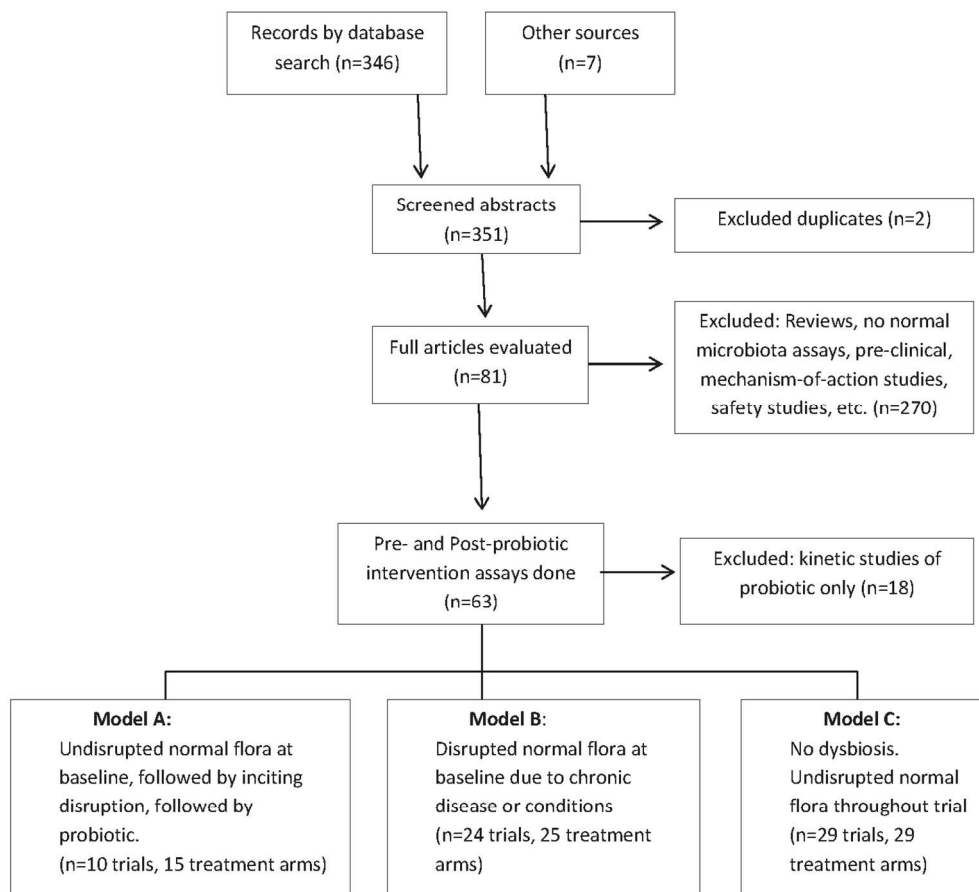
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Model	Type of population enrolled	Dysbiosis at baseline	Time microbiota disrupted	Probiotic or control intervention	Potential outcomes
A	Healthy volunteers or at-risk patients	no	post-baseline	preventive	restoration
B	Patients with active disease at enrollment	yes	pre-baseline	treatment	altered or improved
C	Healthy volunteers	no	not disrupted	preventive	altered

Figure 1. Time sequence of events and three models of study designs determining three different degrees of dysbiosis correction by probiotics  
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Figure 2. Flow-chart of literature review results (1985-2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. Abbreviations: RCT, randomized-controlled trials; MOA, mechanism of action; NM, normal microbiota.  
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Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both. <b>This is a systematic review</b>	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known. <b>Probiotics are promising candidates to prevent or treat disease, but are typically supported by structure/function claims in most countries. The function claim for the restoration of normal microbiota is commonly cited in efficacy trials, but the evidence for this claim has not been examined systematically for all probiotic strains. Differences in study populations and study design effect the type of conclusions that can be drawn. This is the first systematic review and proof of principle of this type of analysis for the function claim of dysbiosis.</b>	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). <b>A comprehensive literature review of the evidence from randomized controlled trials will discuss the strength of the evidence for the restoration or improvement of dysbiosis by specific probiotic strain. The interventions include probiotic or control (typically placebo) given for a specific time enrolled in clinical trials for either the prevention or treatment of disease. All trials which stated some impact on the normal microbiota will be reviewed and analyzed for the ability to document changes in the normal microbiota. The outcomes are the degree of dysbiosis restoration depending upon the study design and type of enrolled participants.</b>	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. <b>Review protocol is described in Methods section of paper. Prospero registration number is: CRD42014007224</b>	5-9
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. <b>Participants: without restriction, any enrolled in clinical trial (adults and pediatrics)</b>	6



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		<p><b>Interventions: all probiotics</b>  <b>Comparisons: controlled (typically placebo)</b>  <b>Outcomes: microbiologic assays of the intestinal flora or microbiota</b>  <b>Study design: required to have pre-intervention (baseline) and post-intervention microbiological assays</b>  <b>Length of follow-up: unrestricted</b>  <b>Language: unrestricted</b>  <b>Publication and years considered: peer-reviewed publications from PubMed (1985-2013, unless otherwise noted), EMBASE, Cochrane Database (1990-2013), CINAHL, AMED, ISI Web of Science (2000-2013). On-line trial registries: (Cochrane Central Register of Controlled trials, MetaRegister of Controlled Trials, and National Institutes of Health.</b></p>	
Information sources	7	<p>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.</p> <p><b>See item above</b></p>	6
Search	8	<p>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</p> <p><b>All probiotics + health claims, structure/function claims, normal microbiota, normal intestinal flora, dysbiosis, pharmacokinetics, metagenomics, dietary supplements, randomized controlled trials, antibiotic-associated diarrhea, Clostridium difficile infections, H. pylori treatments, inflammatory bowel disease, irritable bowel disease, travelers diarrhea, bacterial vaginosis or vaginitis, treatment of pediatric acute diarrhea, healthy volunteer trials and specific probiotic strains. (PubMed)</b></p>	5-6
Study selection	9	<p>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</p> <p><b>Screening:</b>  <b>Eligibility: Must have at least one pre-intervention (baseline) microbiological assay of normal flora or metagenomic analysis and one post-intervention (post-probiotic) assay. Genus and species of probiotic strain(s) provided. Normal microbiota assayed during a randomized, controlled trial.</b>  <b>Excluded: pre-clinical studies, safety studies, reviews, mechanism of action studies, case reports or case series, duplicate reports, unspecified type of probiotics.</b></p>	6-7
Data collection process	10	<p>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</p> <p><b>Pilot data extraction form used modified from standard meta-analysis data extraction form (McFarland and Goh 2013, World J Gastroenterol). Questionable results were queried from original authors of papers.</b></p>	6
Data items	11	<p>List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.</p> <p><b>The timing and type of microbiologic assays of intestinal or vaginal microbiota were collected. As the literature review has a length inclusion period (1985-2013), the types of microbiologic assays have evolved, but all types were included from basic microbiologic assays to metagenomic profiling. The type of probiotic intervention was collected by genus, species and strain (if stated in paper). Types of normal microbiota assays varied by technique. The patient population (healthy volunteers, acute disease or chronic disease) was also collected. Also collected: study size, type of disruptive factor, follow-up duration, stated claims in</b></p>	7-8



## PRISMA 2009 Checklist

		paper.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. <b>Quality of study design (restoration, improvement or no dysbiosis) was used when assessing quality of individual studies. These were then analyzed by stratification on the quality of study design.</b>	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). <b>NA, No pooled RR or DWMs used in this systematic review.</b>	na
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis. <b>NA, No pooled RR or DWMs used in this systematic review.</b>	na

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). <b>All studies with microbiota were included to limit bias, but no measurement for publication bias was done for this systematic review.</b>	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. <b>association of degree of dysbiosis correction with clinical efficacy by probiotic strain(s)</b>	8-9
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. <b>See flow diagram, Figure 2.</b>	25
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. <b>Extracted data were cited in tables. See paper-Tables 1-4.</b>	26-32
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). <b>Presented in Discussion section.</b>	11-15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. <b>Cited in Tables 1-4.</b>	26-32
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. <b>NA, this is a systematic review, not a meta-analysis.</b>	na
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15). <small>For peer review only: <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a></small>	18-19





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		<b>Presented in Discussion section (bias due to study design).</b>	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	15-17
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). <b>Strength of the evidence is provided in the Results section.</b> <b>Relevance to key groups is in the Discussion section.</b>	19, 22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). <b>Provided in Discussion section and “Opportunities for Future Research” section and ‘Strengths and Weaknesses” section.</b>	21-22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. <b>The weight of the evidence for the function claim that probiotics can improve or restore normal microbiota is strong for a few probiotic strains, but in general, more confirmatory studies that are properly timed and designed are required for the majority of probiotic strains.</b>	20-21
<b>FUNDING</b>			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. <b>This review was unfunded.</b>	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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