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

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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 randomised clinical trials of probiotic interventions having microbiological assays. Studies were evaluated following Preferred Reporting Items for Systematic Reviews and Meta-Analyses 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 postdisruptive event and probiotic therapy; model B (alteration) assayed patients with pre-existing disrupted microbiota and then postprobiotic therapy; model C (no dysbiosis) assayed volunteers with no disruptive event prebiotic and postprobiotic. 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 on the enrolled population and the timing of microbiological assays. The functional claim for correcting dysbiosis is poorly supported for most probiotic strains and requires further research.

Trial registration number: PROSPERO (CRD42014007224).

Strengths and limitations of this study

- 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 30 years of research experience in the probiotic field.
- Pooled clinical trials using different study populations.
- Pooled probiotic doses and regimens.
- Indirect evidence linking probiotic strains and dysbiosis.
- Review performed 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 USA, 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 UK, 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.[1–3] In many cases, scientific substantiation of a specific health claim was judged insufficient or based on an indirect effect.[4] 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),[5 6] 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 diarrhoea (AAD).[7 8] 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 'colonisation resistance'.[9 10] The loss of a subpopulation of the normal microbiota, for example, can lead to the loss of the ability to break down fibres and starches into absorbable short chain fatty acids, resulting in high level of undigested carbohydrates, which can trigger diarrhoea.[11] 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,[12 13] vagina,[14 15] respiratory tract,[16 17] and in the buccal cavity.[18–20]

The major challenge to establishing a cause and effect for the improvement of dysbiosis by probiotics is a lack of a standard definition of 'normal' microbiota. There is substantial inter-individual variation of the species of microbes present at different body niches, which also varies by age, geographic area and health status of the host. In addition, a complete accounting of the microbiota is currently impossible, as there are no assays to detect all of $>10^{13}$–10^{14} organisms in the intestines and standard microbial culturing methods miss 75–95% of these organisms.[21 22] The development of metagenomics (cataloguing individual and disease-specific bacterial gene profiles) and the creation of the international Human Microbiome Project ushered in a new era for our understanding of the complexity of these interactions within the body.[23 24] This paradigm shift from culturing to metagenomic analysis has expanded our ability to document shifts in microbial populations to an unparalleled degree, but the interpretation of these shifts continues to be under debate.[25–28] With the advent of these newer metagenomic tools, the role of probiotics in the restoration of normal microbiota is being revisited.[29]

In light of new guidance documents and recommendations, the goal of this systematic review is to determine how claims for the restoration of the normal microbiota and the correction of dysbiosis have been studied using well-designed trials and which probiotic strains have evidence-based data to support these claims.

METHODS

Study objective

To systematically review the literature to analyse the evidence for the claim probiotics can correct dysbiosis of the normal microbiota from randomised controlled trials.

Search strategy

Search terms included: probiotics+health claims, restoring normal microbiota, dysbiosis, normal microbiota, pharmacokinetics, metagenomics, probiotics, dietary supplements, randomised controlled trials, AAD, *Clostridium difficile* infection (CDI), inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), traveler's diarrhoea (TD), eradication of *Heliobacter pylori,* bacterial vaginosis (BV) or vaginitis, treatment of acute paediatric diarrhoea and specific probiotic strains or products. Search strategies were broad-based initially, then narrowed to clinical trials with probiotics.

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 by a single author and rated for inclusion for randomised 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 preclinical 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 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines using a 27-item checklist and flow diagram.[30] A standardised 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 paediatric, 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 specific probiotic strain(s). The secondary outcome is the association between the degree of dysbiosis correction and the net efficacy found from randomised 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 preprobiotic treatment assay and a postprobiotic 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 hybridisation (FISH) or other PCR technique)[8 21 28 31] or by indirect methods (Nugent scores).[15] Nugent scores (ranged 0–10) are used to diagnose bacterial vaginosis (scores ≥7) or normal vaginal microbiota (scores 0–3) based on the quantitated morphotypes of small Gram-negative rods (*Gardnerella 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 categorised 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 (eg, pre-existing chronic disease or active disease) and then post-probiotic therapy; model C (no dysbiosis) assayed volunteers with no disruptive event (before or during the clinical trial) at both preprobiotic and postprobiotic times, as shown in figure 1. 'Recovery' of the normal microbiota is defined as a restoration of the microbiota back to a normal healthy baseline. Recovery may be complete recovery (all assayed microbial levels returned to baseline) or incomplete recovery (partial recovery of some microbial strains, but not all returned to baseline levels). In studies enrolling participants with dysbiosis at baseline (typically due to chronic diseases), it is not possible to show a restoration to normal microbiota levels because a normal, undisturbed microbiota was not present in these types of study participants at the time of enrolment. Therefore, the strongest claim possible for model B designs is for an 'alteration or improvement' of the microbiota. Only data from the probiotic-exposed participants were analysed in this paper. Data from the control groups were used to confirm dysbiosis for participants with chronic diseases or after a disruptive exposure, such as antibiotics or chemotherapy, unaffected by probiotic exposure.[32–34]

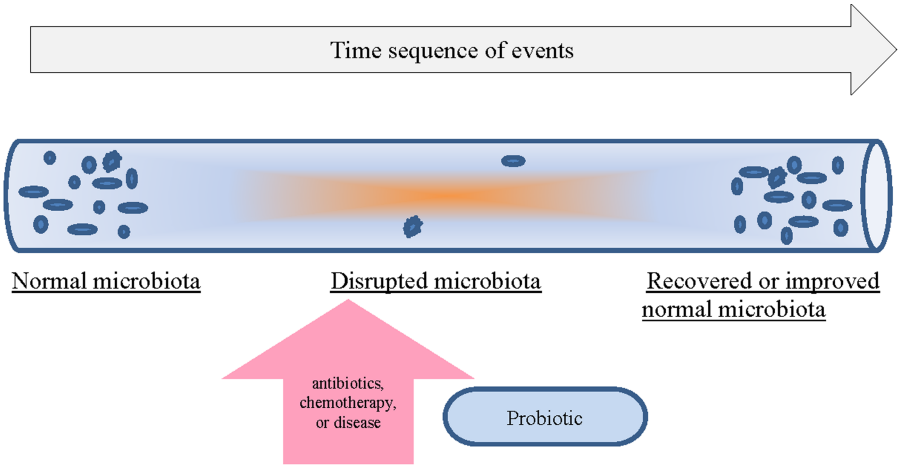
Assessment of methodological strength and quality

The GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system for rating overall study quality will be used for each probiotic strain or type (single strains and mixtures of strains).[35] Recommendation for the support of the claim of each probiotic strain or mixture can be assessed by the overall strength of the evidence ('strong', many randomised controlled trials show significant recovery of the microbiota or 'moderate' only one randomised controlled trial; or 'weak', only case series or reports, limited number of small trials, etc).

Quality of the evidence is based on study design and graded as 'high quality' (well-defined study design for determining restoration with normal microbiota, model A), or 'moderate quality' (disrupted microbiota at baseline, model B), or 'low quality' (no disruptive event occurred, model C). Measurement of publication bias was not assessed for this review, as pooled outcome estimates of efficacy were not carried out, as typical in meta-analysis, but all studies with assays of microbiota were included to limit bias.

Net efficacy rating

To determine if the ability to correct dysbiosis is associated with clinical efficacy, the published literature for randomised controlled trials (RCTs) or meta-analyses of probiotics for various disease indications, including AAD,[5 36 37] CDI,[5 38] IBD,[39] IBS,[40] TD,[41] eradication of *H. pylori*,[36 37] BV[42] and treatment of acute paediatric diarrhoea was reviewed.[43–45] The net rank was calculated by subtracting the number of RCTs showing non-significant or equivalent efficacy from the number of RCTs having significant efficacies. The ranks were categorised as follows:++, ≥2 net RCTs showing significant efficacy;+, net of one RCT showing significant efficacy; 0, equal number of RCTs showing significant and non-significant efficacy results and −, ≥1 net negative or non-



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.

significant RCTs. Probiotics with no RCTs were not ranked.

RESULTS

A review of the literature from 1985–2013 found 353 articles that dealt with probiotic treatments and their potential effect on normal microbiota.

Excluded studies

As shown in figure 2, a total of 272 articles were excluded for the following reasons: reviews (n=116), probiotic efficacy studies with no data on normal microbiota assays (n=54), animal models of probiotics and changes in microbiota (n=38), metagenomic or microbiota methods only (n=17), studies on normal microbiota but with no use of probiotics (n=14), in vitro assays of microbiota (n=10), duplicative reports (n=2) or miscellaneous (n=21), which included probiotic mechanism of action studies, safety studies, duplicative reports, cross-sectional surveys and two with poorly described probiotic interventions.[46 47] A total of 81 full articles were reviewed which mentioned changes in normal microbiota or indicated a health claim for probiotics and effects on normal microbiota.

Probiotic pharmacokinetic studies (n=18) reporting concentrations of probiotic strains before and post-treatment, but not assaying for other species of normal microbiota were excluded. While several studies using this study design claim probiotics had an impact on normal microbiota, type of data generated is pharmacokinetic behaviour of the probiotics themselves and not the normal microbiota. Several studies stated that the normal microbiota was altered because an increase in various bacterial species was observed after the probiotics were given, but the species assayed were those contained in the probiotic product, so an increase is not unexpected. Pharmacokinetic studies have documented that probiotic strains taken orally can survive transit through the intestinal tract with recovery rates in faeces ranging from <1% to 22%.[48 49] These pharmacokinetic studies were excluded from this analysis, as they did not assay other types of normal microbiota not found in the probiotic product.

Included studies

Of the 63 included clinical trials, five trials had multiple treatment arms, which resulted in a total of 69 treatment arms for analysis. Engelbrektson *et al*[50] tested a mixture of five probiotic strains in volunteers exposed to antibiotics and also tested a mixture of four probiotic strains in healthy volunteers with no antibiotic exposure. Zoppi *et al*[51] had eight different treatment arms in his study,

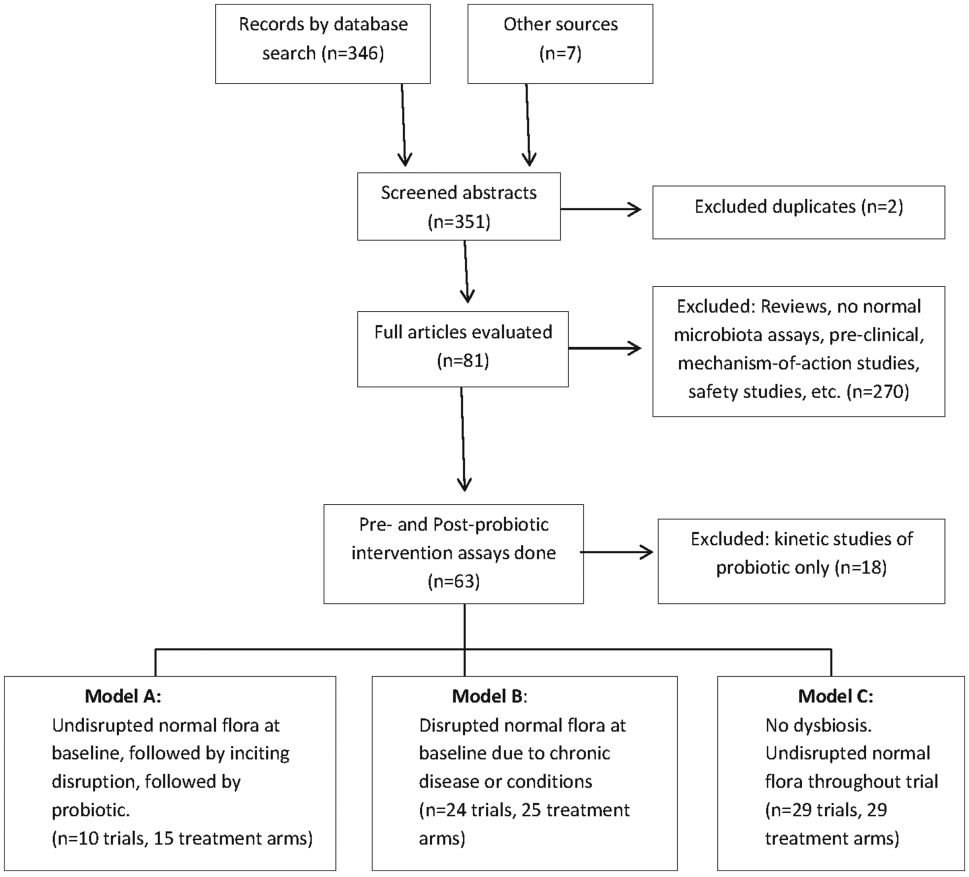


Figure 2 Flow chart of literature review results (1985–2013) of included and excluded studies for the restoration or improvement of normal microbiota by probiotics. RCT, randomised-controlled trials; MOA, mechanism of action; NM, normal microbiota.

and probiotic arms were included in our analysis (*Saccharomyces boulardii* alone and *Lactobacillus rhamnosus GG* alone), a mixture of two probiotics (*L. acidophilus* and *Bifido bifidum*) and a mixture of three probiotic strains (*L. acidophilus, L. rhamnosus* and *B. bifidum*). Orrhage *et al*[52] had two treatment arms (*Bifido longum* alone and a mixture of *B. longum* and *L. acidophilus*). Larsen *et al*[53] tested two single probiotics (*B. lactis* and *L. acidophilus*) in separate treatment arms. Lidbeck *et al*[54] gave either enoxacin or clindamycin and randomised patients to either *L. acidophilus* or placebo.

Normal microbiota assay methods

Of the 69 treatment arms that did normal microbiota assays, diverse methods were used to profile the microbiota. Many studies used only standard microbiological culture assays (37, 54%), while others (28, 40%) used techniques to detect non-cultivatable bacterial strains, which included metagenomic assays (FISH, TRFLP, 16 s rRNA sequencing) or other PCR techniques. Some studies (4, 6%) used an indirect measure of normal microbiota, using the Nugent score to diagnose bacterial vaginosis, which relies on Gram stain of the vaginal secretions, vaginal pH and symptoms to characterise if normal microbiota is present or absent.[15]

Probiotic strains

In the 69 treatment arms, most (36, 52%) used a single strain of probiotic, while 14 (20%) tested a mix of two probiotic strains and 19 (28%) tested a mix of three or more probiotic strains. The distribution of single versus multiple strain probiotics did not significant vary by the model of study design (χ^2_2=2.3, p=0.32). Of the 15 restorative (model A) study arms, 47% used a single strain of probiotic and 53% used multiple strains. Of the 25 treatment arms with disrupted microbiota at baseline (model B), 44% used a single strain and 56% used multiple strains. Of the 29 study arms with undisrupted microbiota (model C), 62% used a single strain and 38% used multiple strains.

Normal microbiota restoration model (model A)

Only 10 studies (with 15 treatment arms) using model A to determine restoration of the microbiota were found (table 1).[32 34 50–52 54–58] The type of enrolled participants varied from healthy volunteers to children with untreated respiratory infections, to paediatric cancer patients. For participants with acute infections or cancer, baseline assays were performed prior to the disrupting agent (antibiotics or chemotherapy). The number of participants given probiotics averaged 20/study and

ranged from 5 to 83. In 93%, the disruptive factor was antibiotic exposure and in one study, chemotherapy caused the microbiota disruption. Only 8 (53%) of the study arms did an assay during a 1–8 weeks follow-up period after the probiotic was discontinued.

Analysis of the probiotic strain(s) separately found only two probiotic products with more than one randomised controlled trial. The probiotic mix of *L. acidophilus* and *B. bifidum* showed a complete restoration in one study, but only a partial recovery in the other (Strength: strong, Quality: high). The probiotic mix of *L. acidophilus* (2 strains) with *B. bifidum* and *B. 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 (*B. longum*, *Clost. butyricum*, *L. acidophilus*, mix of *L. acidophilus* with *L. paracasei* and *B. lactis* and the mix of *L. acidophilus* with *L. paracasei* and *B. bifidum* and two strains of *B. 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 (*B. breve* and a mix of *L. acidophilus* and *B. longum*; Strength: moderate, Quality: high). In summary, 10 of 12 (83%) of the probiotic products showed complete or partial restoration of the normal microbiota.

Of the 11 probiotic products with claims of 'restores or improves normal microbiota', 10 (91%) were supported by this review, but only seven showed complete restoration and five had partial restoration of the microbiota (table 1). The mixture of *L. acidophilus* and *B. longum* did not show any changes in the microbiota. Wada *et al*[32] claimed *B. breve* 'enhanced intestinal anaerobes', but this was only compared to the placebo group. 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 weeks follow-up in either the probiotic or the placebo group compared to baseline microbiota levels.

Disrupted normal microbiota at baseline studies (model B)

Twenty-four studies (with 25 treatment arms) used model B that enrolled participants with a pre-existing disrupted microbiota related to ongoing disease or conditions (table 2).[33 53 59–80] The number of participants given probiotics averaged 23±16/study and ranged from 7 to 83 participants. The types of pre-existing factors that disrupted the microbiota included atopic dermatitis patients, allergies, cirrhosis, bacterial vaginosis, irritable bowel syndrome, inflammatory bowel disease (ulcerative colitis and pouchitis), idiopathic diarrhoea, enteral feeding, short-bowel syndrome and colon cancer. Only 10 (40%) of the study arms did an assay during the post-probiotic follow-up period.

Three of the probiotics had multiple clinical trials to support the claim of an improvement in the microbiota due to the probiotic. *S. boulardii* was used in two trials either with enteral fed patients or patients with active diarrhoea and found an improvement in the habitual microbiota in the patients with active diarrhoea,[66] but only showed indirect evidence of short-chain fatty acid changes in the other study[65] (Strength: strong, Quality: moderate). A mix of four probiotic strains (2 strains of *L. rhamnosus*, *P. freudenreichii*+*B. breve*) showed improved microbiota in two clinical trials[74 75] (Strength: strong, Quality: moderate). Of four clinical trials testing a mixture of seven probiotic strains, two showed no significant change in microbiota,[77 78] one showed more anaerobes postprobiotic treatment[79] and one found a reduction in bacteroides species[80] (Strength: strong, Quality: moderate). Three clinical trials determined there were no significant changes due to *Lactobacillus plantarum* 299v[62–64] (Strength: strong, Quality: moderate). Of those probiotics with only one supporting clinical trial (Strength: moderate, Quality: moderate), two single probiotic strains (*E. coli* Nissle and *L. casei rhamnosus*) and five different mixtures of probiotic strains support the claim that the probiotic alters the microbiota (table 2). In summary, 10 of 18 (56%) probiotic products altered or improved microbiota in individuals with pre-existing disease.

Of the 25 treatment arms, the paper's claim was confirmed in 14 (56%) of the studies. There was no significant change in the microbiota due to the probiotic in nine treatment arms and only an alteration of the microbiota in five others (table 2). Our review disagreed with the claimed outcomes in 11 (46%) of the other treatment arms. In seven treatment arms, it was claimed the tested probiotic 'restored normal microbiota', but it is uncertain how this conclusion was reached, since there was no time when a normal undisrupted microbiota was present. Of the seven studies that claimed their probiotic 'restored' normal microbiota, our analysis determined none were capable of documenting restoration, but it is confirmed probiotics improved or altered the microbiota in these studies. Four studies claimed the probiotic 'altered or improved' normal microbiota, but this review found no significant differences when postprobiotic and baseline assays were compared for the probiotic groups. Girard-Pipau *et al*[65] 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. However, when the analysis is restricted to trends observed in the probiotic group only, no significant differences were observed in preprobiotic versus postprobiotic microbiota profiles. Venturi *et al*[77] concluded that the mix of seven probiotic strains enhanced the concentration of some beneficial strains in the intestines. However, the only strains having a significant increase were those contained in the probiotic mix, and not specifically normal

Table 1 Evidence-based data for restoration of normal microbiota (NM) for 12 probiotics from 10 studies (15 treatment arms; model A)

Probiotic*	Reference	Number treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treatment (weeks)	Claims stated in papers	Evidence-based claim
Bifido breve	Wada *et al*[32]	19	FISH	Paediatric cancer patients	Chemotherapy	8	Enhances anaerobes	No change
Bifido longum BB536	Orrhage *et al*[52]	10	Culture	Healthy volunteers	Clindamycin	0	Restores	Restores
Clostridium butyricum MIYAIRI	Seki *et al*[34]	83	Culture	Paediatric respiratory or GI infections	Antibiotics	0	Restores	Restores
Lactobacillus acidophilus NCFB1748	Lidbeck *et al*[54]	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
Lactobacillus rhamnosus GG	Zoppi *et al*[51]	7	Culture	Paediatric respiratory infections	Ceftriaxone	0	Partially corrects	Partially restores
Saccharomyces boulardii lyo	Zoppi *et al*[51]	6	Culture	Paediatric respiratory infections	Ceftriaxone	0	Improves	Partially restores
L. acidophilus+Bifido bifidum	Black *et al*[55]	10	Culture	Healthy volunteers	Ampicillin	2	Recovers more rapidly	Restores
	Zoppi *et al*[51]	7	Culture	Pediatric respiratory	Ceftriaxone	0	Less change	Partially restores
L. acidophilus 1748*+B. longum* BB536	Orrhage *et al*[52]	10	Culture	Healthy volunteers	Clindamycin	0	No change	No change
L. rhamnosus+L. bifidus+L. acidophilus	Zoppi *et al*[51]	7	Culture	Paediatric respiratory infections	Ceftriaxone	0	Partially corrects	Partially restores
L. acidophilus 1748 *+Lactobacillus paracasei* F19*+Bifido lactis* Bb12	Jernberg *et al*[56]	4	Culture PCR TRFLP	Healthy volunteers	Clindamycin	2	Restores	Restores
L. acidophilus CUL60*+L. acidophilus* CUL21*+B. bifidum* CUL17*+Bifido animalis lactis*	Madden *et al*[57]	15	Culture	*Helicobacter pylori+*	Amoxicillin +metronidazole	2	Restores	Restores
	Plummer *et al*[58]	76	Culture	*H. pylori+*	Amoxicillin +clarithromycin	2	Restores more rapidly	Partially restores
L. acidophilus NCFM+L. paracasei Lpc-37*+B. bifidum* Bb02*+B. lactis* Bi-04*+B. lactis* Bi-07	Engel-brektson *et al*[50]	20	Culture PCR TRFLP	Healthy volunteers	Augmentin	2	Restores	Restores

*Including strain (when reported).
GI, gastrointestinal.

Table 2 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 (model B)

Probiotic*	Reference	Number 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
Bifido breve M-16V	Van der Aa *et al*[59]	46	FISH	Atopic dermatitis	0	Modulates NF	No change	–
Bifido lactis Bi-07	Larsen *et al*[53]	17	PCR	Atopic dermatitis	0	No change	No change	–
Bifido longum BB536	Odamaki *et al*[33]	22	TRFLP PCR	Cedar pollen allergy	4 weeks	Maintains NF	No change	–
Escherichia coli Nissle	Lata *et al*[60]	22	Culture	Liver cirrhosis	0	Restores	Improves	More *Bifido* and *Lactobacillus*
Lactobacillus acidophilus 700396	Larsen *et al*[53]	17	PCR	Atopic dermatitis	0	No change	No change	–
Lactobacillus casei rhamnosus Lcr35	Petricevic and Witt[61]	83	Nugent scores	Bacterial vaginosis	4 weeks	Restores	Improves	Improved Nugent scores
Lactobacillus plantarum 299v	Nobaek *et al*[62]	25	Culture	IBS	4 weeks	No change	No change	–
	Klarin *et al*[63]	17	Culture	Enterally-fed	0	No change	No change	
	Klarin *et al*[64]	22	Culture	Antibiotics	0	No change	No change	
Saccharomyces boulardii lyo	Girard *et al* [65]	10	Culture	Enterally-fed	9 days	Alters NF	No change	–
	Swidsinski *et al*[66]	20	FISH	Active diarrhoea	3 weeks	Improves	Improves	More 'habitual microbiota'
L. rhamnosus GR-1+*Lactobacillus fermentum* RC14	Reid *et al*[67]	33	Nugent scores	Bacterial vaginosis	2 weeks	Restores	Improves	Improved Nugent scores
L. rhamnosus GR-1+*L. fermentum* RC14	Reid *et al*[68]	31	Nugent scores and culture	Bacterial vaginosis	30 days	Restores	Improves	Improved Nugent scores
Lactobacillus plantarum 8PA3 +*Bifido bifidum*	Kirpich *et al*[69]	32	Culture	Colon cancer	0	Restores	Improves	More *E. coli* and enterococci
L. rhamnosus GR1+*Lactobacillus reuteri* RC14	Hummelen *et al*[70]	23	Nugent score	Bacterial vaginosis	0	No change	No change	–
L. casei Shirota+*B. breve* BBG01	Uchida *et al*[71]	4	Culture	Short bowel syndrome	0	No change	No change	–
L. brevis CD2+*Lactobacillus salivaris* FV2+*L. plantarum* FV9	Mastromarino *et al*[72]	19	Nugent score	Bacterial vaginosis	2 weeks	Restores	Improves	Improved Nugent scores
L. paracasei Lpc37+*L. acidophilus* 74-2+*Bifido animalis* DGCC420	Roessler *et al*[73]	30	PCR	Atopic dermatitis	0	No change	No change	–
L. rhamnosus GG+*L. rhamnosus* Lc705+*Propionibacterium freudenreichii shermanii* JS+*B. breve* Bb99	Kajander *et al*[74]	41	PCR	IBS	0	Restores	Improves	Improved similarity index
	Lyra *et al*[75]	22	PCR	IBS	0	Alters	Alters	More clostridia and *Ruminococcus*

Continued

Table 2 Continued

Probiotic*	Reference	Number 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
L. acidophilus 4356+*L. plantarum* 14917+*L. rhamnosus* 7469+*B. bifidum* 2952	Wong *et al*[76]	7	PCR	Liver disease	0	Improves	Alters	Less firmicutes, more bacteriodetes
L. acidophilus+*L. paracasei*	Venturi *et al*[77]	20	Culture	Ulcerative colitis	15 days	Enhances	No change	–
+*Lactobacillus delbrueckii spp bulgaricus*+*L. plantarum*+*B. longum*+*B. infantis*+*B. breve*	Brigidi *et al*[78]	10	Culture and PCR	IBS	10 days	No change	No change	–
	Kuhbacher *et al*[79]	10	FISH	Pouchitis	0	Altered richness	Altered	More anaerobes
	Ng *et al*[80]	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.
IBS, irritable bowel syndrome; FISH, fluorescence in situ hybridisation analysis; NM, microbiota.

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*[59] claimed that *B. breve* 'successfully modulates the intestinal flora', but no significant changes were observed in the probiotic group when comparing the baseline to the postprobiotic levels. Odamaki *et al*[33] did show an increase in *Faecalibacterium* spp and *Bacteroides fragilis* spp at the end of *B. longum* BB536 treatment, but the same increase was also observed in the placebo group.

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.[14 49 50 81–106] The average number of participants 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%. Fujiwara *et al*[84] cultured seven healthy volunteers and found enterobacteriaceae and *Clostridial* species post-*B. longum* was reduced by 10^1/g compared to baseline ($p<0.03$), but no other changes in the microbiota were detected. Karlsson *et al*[94] found a significant increase in intestinal diversity in nine male volunteers with atherosclerosis given *L. plantarum* 299v, but because terminal restriction fragment length polymorphism assays were used instead of cultures for bacterial species, the specific changes in the microbiota species could not be determined. Yang and Sheu cultured 63 children (55% with *Helicobacter pylori*) given a yogurt with *L. acidophilus* and *B. lactis* but only found a decrease in *E. coli* counts in the *H.pylori* negative children subgroup, no significant changes in normal microbiota was found in the *H. pylori*-positive children.[100] Kubota *et al*[103] assayed 29 participants with Japanese cedar pollen allergy and found milk fermented with *L. rhamnosus* GG and *L. gasseri* TMC0356 suppressed microbiota changes (less intestinal profile changes), but could not determine specific bacterial species changes due to the type of assay used (FISH and TRFLP).[103] In summary, only 4 of 19 (21%) probiotic products altered microbiota in healthy individuals who had no disruptive event.

Of the seven studies that claimed their probiotic(s) 'restored or altered' the normal microbiota, only four claims were confirmed. Sierra *et al*[96] claimed *Lactobacillus salivarius* given to 20 healthy adults 'improved gut microbiota', but only increased levels of Lactobacilli were found and no other changes in normal microbiota species were detected. The only other evidence was indirect from changes observed in immune parameters. He *et al*[99] claimed a mixture of *B. longum* and *B. animalis* 'modified' microbiota, but changes were seen only during the yogurt administration and not after the 1 week follow-up period. Vitali *et al*[14] claimed that the mixture of four lactobacilli strains and three bifidobacteria strains 'modulated vaginal

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	Number treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treat-ment	Claims stated in papers	Evidence-based claim
Bifido animalis lactis DN173010	Rochet *et al*[49]	12	FISH	Healthy	None	10 days	No change	No change
	Oswari *et al*[81]	160	PCR	Volunteers	None	6 months	No change	No change
Bifido bifidum	Langhendries *et al*[82]	20	Culture	Healthy volunteers	None	0	No change	No change
Bifido longum	Benno and Mitsuoka[83]	5	Culture	Healthy volunteers	None	0	No change	No change
	Fujiwara *et al*[84]	7	Culture		None	30 days	Alters	Alters
	Harmsen *et al*[85]	14	FISH		None	0	No change	No change
Lactobacillus casei ND114001	Guerin *et al*[86]	12	Culture	Healthy volunteers	None	1 weeks	No change	No change
	Rochet *et al*[87]	12	FISH		None	10 days	No change	No change
	Rochet *et al*[88]	7	FISH		None	0	No change	No change
Lactobacillus johnsonii La1	Brunser *et al*[89]	32	Culture and FISH	Healthy volunteers	None	2 weeks	No claim	No change
Lactobacillus plantarum 299v	Goossens *et al*[90]	11	Culture	Healthy	None	3 weeks	No change	No change
	Goossens *et al*[91]	32	Culture	Healthy	None	4 weeks	No change	No change
	Goossens *et al*[92]	15	Culture	Colonic	None	0	No change	No change
	Berggren *et al*[93]	33	Culture	Polyps	None	0	No change	No change
	Karlsson *et al*[94]	9	TRFLP	Healthy, atherosclerosis	None	0	Alters	Alters
Lactobacillus rhamnosus GG	Gueimonde *et al*[95]	29	PCR	Healthy volunteers	None	0	No change	No change
Lactobacillus salivarius CECT5713	Sierra *et al*[96]	20	Culture	Healthy volunteers	None	0	Improves	No change
Saccharomyces boulardii lyo	Vanhoutte *et al*[97]	30	PCR	Healthy volunteers	None	0	No change	No change
B. animalis+B. longum	Zhong *et al*[98]	11	FISH	Healthy volunteers	None	7 days	No change	No change
	He *et al*[99]	11	FISH			7 days	Modifies	No change
L. acidophilus+B. lactis	Yang and Sheu[100]	63	Culture	Healthy but 55% *H. pylori+*	None	0	Restores	Alters
L. rhamnosus GG+*B. longum* Bb536	Mah *et al*[101]	20	FISH	Healthy neonates	None	6 months	No change	No change
L. rhamnosus GG+*B. lactis* Bb12	Rafter *et al*[102]	38	Culture	Patients with colon cancer or at risk	None	0	No change	No change

Continued

Table 3 Continued

Probiotic*	Reference	Number treated with probiotic	Type of assay for NM	Enrolled population	Type of disrupting factor	Follow-up post-treat-ment	Claims stated in papers	Evidence-based claim
L. rhamnosus GG+*Lactobacillus gasseri* TMC0356	Kubota *et al*[103]	14	Culture FISH TRFLP	Healthy, allergy patients	None	0	Suppressed changes	Alters
L. paracasei B21060+*L. paracasei* B21070+*L. gasseri* B21090	Morelli *et al*[104]	12	Culture	Healthy volunteers	None	3 days	No claims	No change
L. acidophilus 1748+*L. paracasei* F19+*B. lactis* Bb12	Sullivan *et al*[105]	15	Culture	Chronic fatigue patients	None	4 weeks	No change	No change
L. rhamnosus 271+*L. acidophilus* NCFM+*L. paracasei* 114001+*B. animalis* 1017	Engelbrektson *et al*[50]	22	Culture TRFLP PCR	Healthy volunteers	None	2 weeks	No change	No change
B. animalis lactis+*Lactobacillus delbrueckii* I-1632+*L. delbrueckii I*-1519+*L. lactis cremoris*	McNulty *et al*[106]	7	PCR	Healthy twins volunteers	None	4 weeks	No change	No change
L. acidophilus+*L. paracasei*+*L. delbrueckii spp bulgaricus*+*L. plantarum*+*B. longum*+*B. infantis*+*B. breve*	Vital *et al*[14]	15	PCR	Healthy pregnant volunteers	None	0	Modulates	No change

*Including strain (when reported).
FISH, fluorescence in situ hybridisation analysis; TRFLP, terminal restriction fragment length polymorphism analysis.

microbiota', but the only significant changes were due to an increase in the bacterial species contained in the probiotic mixture.

Of the probiotics supported by multiple clinical trials (*B. animalis*, *B. longum*, *L. casei*, *L. plantarum* 299v, the mixture of *B. animalis and B. lactis*), 13 of the trials (87%) support there is no significant change in normal microbiota if the microbiota is not disrupted (Strength: strong, Quality: low).

Association of clinical efficacy and normal microbiota restoration

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 seven 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 paediatric diarrhoea (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 paediatric diarrhoea (ranked++: 10 RCTs with significant efficacy and one with non-significant findings).

Efficacy trials were not carried out as frequently for probiotics shown to only have the ability to alter or improve, but not restore, the microbiota after dysbiosis. Of nine probiotics that can alter the microbiota, 6 (67%) have supporting RCTs for at least one disease, but the diversity of investigated diseases was more limited. *L. casei* had moderate net strength for AAD and bacterial vaginosis, but was neutral for the ability to eradicate *H. pylori* and other disease indications were not tested in RCTs with *L. casei*. The probiotic mixture of *L. reuteri* and *L. fermentum* has strong evidence for bacterial vaginosis, but not for any other disease indications listed in table 4.

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. *B. lactis* and the mixture of *L. rhamnosus* and *L. reuteri* had net neutral rankings for efficacy for the treatment of acute paediatric diarrhoea. The other four probiotic products with no effect on normal microbiota lacked any RCTs for clinical efficacy. Studies with *Bacillus clausii* did not assay for normal microbiota and had non-significant trial results for *H. pylori* eradication and the treatment of paediatric diarrhoea.

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 (*B. clausii*, *B. infantis*, *L. reuteri*, *L. acidophilus+L. helveticus*, *L. acidophilus+L. casei* and *L. acidophilus+B. 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 organisations, including the World Gastroenterology Organization[107] and the American Society for Nutrition.[2] The US 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.[108] 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.[3] 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

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 paediatric diarrhoea
Restores microbiota										
Clostridium butyricum MIYAIRI	Yes	ND	–					–		
Lactobacllius. acidophilus+Bifido bifidum	Yes	ND	0	–						
L. acidophilus 1748+*Lactobacllius paracasei* F19+*Bifido lactis* Bb12	Yes	ND				–				
Bifido longum	Yes	No			–	+				
L. acidophilus+L. acidophilus+B. bifidum+B. animalis	Yes	ND								
L. acidophilus+L. paracasei+B. lactis (2)	Yes	No								
Saccharomyces boulardii lyo	Partial	Yes	++	++	++	0	+	–		++
L. rhamnosus GG	Partial	ND	–	–	–	0	0	–	0	++
L. acidophilus	Partial	No	++			++	–	–	+	0
L. acidophilus+L. bifidus+L. rhamnosus	Partial	ND								
Alters microbiota										
Escherichia coli Nissle	ND	Yes			–					+
L. casei (DN114001 or Lcr35)	ND	Yes	+					0	+	++
L. rhamnosus GR1+*Lactobacllius fermentum* RC14	ND	Yes							++	
L. plantarum 8PA3+*B. bifidum*	ND	Yes								
Lactobacllius rhamnosus GG+*L. rhamnosus* Lc705+*P. freudenreichii shermanii* JS+*Bifido breve* Bb99	ND	Yes				++				
L. acidophilus+L. plantarum+L. rhamnosus+B bifidum	ND	Yes								
Lactobacllius brevis CD2+*Lactobacllius. salivarus* FV2+*L. plantarum* FV9	ND	Yes							**+**	
L. acidophilus+L. paracasei+Lactobacllius delbrueckii spp. bulgaricus+L. plantarum, Bifido longum, Bifido infantis, Bifido breve	ND	Yes	–		**++**	**+**				**++**
No effect on microbiota										
Bacillus clausii	ND	ND						–		–
L. plantarum 299v	ND	No	–	–		–				
B. lactis	ND	No	**+**							**0**
B. breve	No	No								
L. acidophilus+B. longum	No	ND								
L. rhamnosus 19070-2+*L. reuteri DSM*	ND	No								**0**
L. casei+B. breve	ND	No								
L. paracasei+L. acidophilus+B animalis	ND	No								
Pharmacokinetic only										
L. reuteri 55730	ND	ND								**+**
L. johnsonii La1	ND	ND			–			**+**		
L. salivarius UCC4331	ND	ND				–				
B. infantis 35624	ND	ND				**0**				
B. bifidum MIMBb75	ND	ND				**+**				
L. rhamnosus+B. longum	ND	ND								

*Including strain (when reported).
†Rank (bold values):**++**, ≥2 net randomised controlled trials (RCTs) with significant protective efficacy;+, only one net protective RCT; 0, equal number of significant and non-significant RCTs;–, ≥1 net non-significant RCT. Blank indicates no RCT performed for the disease indication.
AAD, antibiotic-associated diarrhoea; Acute Ped Diar, treatment of acute paediatric diarrhoea; BV, bacterial vaginosis; CDI, *Clostridium difficile* infections; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome;ND, not determined; TD, traveler's diarrhoea.

define a standard normal microbiota profile, the EFSA recommends functional claims for the restoration of normal microbiota should document a recovery of healthy microbiota and be accompanied by a beneficial physiological or clinical outcome.[3] In addition, because the efficacy and mechanisms are strain-specific and may vary by probiotic strain, the evidence must be analysed for each probiotic product individually.[5 6 9 109–112]

An underappreciated finding was the influence that study design and study populations have on the interpretation of study outcomes. In the literature, five different types of study designs are commonly found relating to probiotics. The most common study type is a randomised controlled trial testing the efficacy and safety outcomes in patients, but these trials did not typically document the impact of the probiotic on the normal microbiota. The second most common type of study design is pharmacokinetic studies (documenting recovery of oral dose of probiotic or increase in probiotic strains post-treatment compared to pretreatment or clearance of the probiotic). Even though these kinetic studies did not assay for non-probiotic strains, some extrapolated their results and concluded some effect or improvement of the normal microbiota was observed by their probiotic.[19 111] These two first types of study designs do not support evidence-based conclusions for the restoration or alteration of the normal microbiota and were excluded from this review.

Three types of study designs are appropriate for the study of dysbiosis. The first type of study design had normal microbiota assayed at least twice (at baseline, which was before exposure to a disruptive event or probiotics and then again during or postprobiotic treatment) to show actual recovery of assayed normal microbiota back to healthy baseline levels. The second type of study design started with inappropriate baselines (baseline samples taken after normal microbiota had been disrupted by chronic disease). For patients with established chronic diseases, there is no 'normal microbiota' baseline in either the probiotic or the control group. Even if baselines are taken during remission, the microbiota may still be impacted by chronic disease or acute diarrhoea. Studies of probiotics in chronic diseases or acute disease typically report on 'pre-probiotic treatment' and 'post-probiotic treatment' and may show significant shifts in microbial species, but it is uncertain if this reflects a true re-establishment of normal microbiota profiles. The third type of study design enrolled healthy volunteers, who were not challenged with antibiotics (so no normal microbiota disruption occurred) and show only the effect of probiotics on a healthy microbiota (typically mild or no effects). Control groups were not required for our assessment of the impact of probiotics on microbiota, but control groups can document the degree normal microbiota is disrupted by inciting agents (antibiotic, disease onset, etc).

Five single strain probiotics (*B. longum*, *Clost. butyricum*, *L. acidophilus*, *L. rhamnosus* and *S. boulardii*) and five probiotic mixtures ((*L. acidophilus+B. bifidum*), (*L. rhamnosus+L. bifidus+L. acidophilus*), (*L. acidophilus+L. paracasei+B. lactis*), (*L. acidophilus*, 2 strains, *B. bifidum*, *B. animalis*) and (*L. acidophilus+L. paracasei+B. bifidum+2 strains of B. lactis*)) documented either complete or partial recovery of normal microbiota (model A). Only two probiotic mixtures ((2 strain mixture: *L. acidophilus +B. bifidum*) and (4 strain mixture: *L. acidophilus*, 2 strains, *B. bifidum*, *B. animalis*)) were supported by a confirmatory study. Evidence that probiotics may alter or improve normal microbiota (model B) was found for three single strain probiotics (*E. coli* Nissle, *S. boulardii* and *L. casei rhamnosus*) and seven mixtures of 2–7 probiotic strains. Of these 10 probiotics finding alteration of the microbiota, only three had multiple trials: *S. boulardii*, a four strain mixture (2 strains of *L. rhamnosus+P. freudenreichii+B. breve*), and a seven strain mixture (4 lactobacilli and 3 bifidobacteria strains), but only one had consistent results showing improvements in the microbiota.[74 75] Clearly, more than one study is needed to confirm the impact of a probiotic on the normal microbiota. Of the 19 probiotic strains (or mixtures) studied in healthy volunteers who were not exposed to disruptive factors (model C), no change in the normal microbiota was observed for 79%, indicating the robustness of the microbiota.

Improvement in the normal microbiota by specific probiotic strains seemed to be associated with better clinical end points. Within eight common diseases typically treated with probiotics, more trials with significant efficacy were associated with probiotic strains shown to restore the normal microbiota and only one trial with significant efficacy was found for probiotics that did not alter the microbiota. However, few probiotics had efficacy trials for all eight diseases and many did not have any efficacy trials.

Some probiotics which have published efficacy trials for various diseases did not have studies investigating the effect of the probiotic on normal microbiota: *B. clausii*, *B. infantis*, *L. brevis*, *L. reuteri*, mix of two strains (*L. acidophilus+L. helveticus*), mix of two strains (*L. acidophilus+L. casei*) or (*L. acidophilus+B. animalis*), mix of four strains (*L. rhamnosus* (two strains), *P. freudenreichii+B. animalis*)) and mix of seven 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 standardisation of health or function claims, the use of proper study designs and the challenge of defining biomarkers for a 'healthy microbiota'.[3 29 112] Donovan *et al*[2] recommends that health claims for probiotics be supported by well-conducted human trials in the targeted population. These reviews also recommend that gut biomarkers need to be correlated with clinical endpoints, however

none of these reviews attempted to do so.[29 112] No prior review has attempted to analyse the association between probiotic strains and their impact on normal microbiota by stratifying on the quality of study design.[111] This review addressed these concerns by analysing 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 microbiological 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.[21 22] 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. Characterisation of the microbiota is a complex issue and a comprehensive accounting of all the bacterial and fungal strains in the body is beyond our current capabilities. Therefore, any studies of changes to the microbiota are incomplete at best, but general trends in bacterial phylotypes can be documented using DNA probes and metagenomic techniques. Differential detection bias may be present due to the variety of assays used in these studies and should be accounted for in future studies.

Another suggestion for future studies is to include an appropriate follow-up time period postprobiotic administration. Fewer than half of the reviewed trials did assays for normal microbiota during an appropriate follow-up period. As it has been shown that recovery from a disrupting factor can be prolonged (typically 8 weeks),[7 8] and studies that failed to find microbiota recovery might have detected a return to normal baseline levels if a sufficiently long time was given for the recovery to have occurred. Future studies should strive to allow time for the restoration of the normal microbiota to occur.

As the effects of probiotics are strain specific, and many studies typically only report the genus and species of the tested probiotic, future reports should include a complete description of the probiotic to the stain level.[5 112]

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: a single author reviewed and extracted the literature, pooling trials from different populations (adult vs paediatric) 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 carried out. Another limitation is the current lack of a standard definition of what comprises a 'normal microbiota'. The constituents of the microbiota vary by individual, by age, geographic location and health status of the host. Current microbiological techniques are improving, but cannot detect all species present in the host.

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 twofold: 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. These types of issues should be considered for healthcare policymakers and researchers for future studies and for creating guidelines for health/function claims.

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